



FINAL REPORT



National Agricultural Innovation Project (Indian Council of Agricultural Research)

Value Chain on production of food grade nutraceuticals for use as natural antioxidants and food colorants



**Division of Agricultural Chemicals
Indian Agricultural Research Institute, New Delhi-110 012**

2014

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**Value chain on production of food-grade nutraceuticals
for use as natural antioxidants and food colorants**



**Division of Agricultural Chemicals
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FOREWORD

Nutraceutical revolution began in the early eighties in the developed countries and later spread all over the world. The international market for nutraceuticals is still expanding and at present the global size of the industry is over 125 billion USD. With increasing awareness about health and the risk of lifestyle disorders, consumers are looking at nutraceuticals such as natural antioxidants and food colorants as effective health benefitting products. Usually obtained from a food source, these are sold as concentrates or purified components or as ingredients of functional foods. At present Indian nutraceutical market is in its infant stage having its share in this market less than 1%. Recognizing the huge potential of nutraceuticals, ICAR granted a NAIP sub-project “Value chain for the production of food grade nutraceuticals for use as natural antioxidants and food colorants” to Indian Agricultural Research Institute, New Delhi in 2009. The project aimed at developing technical know-how for processes/ products and protocols for efficient extraction, processing, purification, analysis (quality control) of food grade nutraceuticals from vegetables like carrot, tomato and capsicum, fruits like jamun, non-food crop like *Stevia* and a micro-algae- *Spirulina*.

I am extremely happy to learn about the completion of the NAIP sub-project on December 31, 2012. The final report highlighting the salient achievements has been prepared incorporating research contributions of all the consortium partners. Successful implementation of the project has led to development of technologies for production of anthocyanin concentrates from black carrot and jamun, capsanthin concentrate from paprika (chili), lycopene concentrate from tomato, stevioside concentrate from *Stevia* and phycocyanin concentrate from *Spirulina*. These products have been used to produce time released nano sized formulations, which are bioavailable and stable. Nutraceutical enriched purple/golden breads, muffins, aonla candy, juices, RTS beverages etc. have also been developed. MoUs for transfer of technology for some of these products have been signed with two industries namely Ozone Biotech, Faridabad and Pratistha Industries, Secundrabad. Efforts made by the Consortium Principal Investigator Dr Suresh Walia and his team of CCPIs, and Co-PIs deserve enormous appreciation. I am hopeful that in the foreseeable future products based on the outcome of the project will be commercially available thus benefitting the farmers, industry, other stakeholders and general public in large.

Feb 15, 2014
New Delhi


(H. S. Gupta) 15/2/14
Director & Consortium Leader

PREFACE

It gives me immense pleasure in presenting the final report on the NAIP sub-project. “Value chain for the production of food grade nutraceuticals for use as natural antioxidants and food colorants” (Code 50:56). The World bank funded NAIP sub-project was awarded to Indian Agricultural Research Institute (IARI) New Delhi in 2009 to focus attention on developing technical know-how and protocols for efficient extraction, processing, purification, analysis, and quality control of food grade nutraceuticals and functional foods from food and non-food crops. Besides IARI (lead centre) comprising of the Divisions of Agricultural Chemicals, Microbiology and Post Harvest Technology, other consortium partners included Dr YS Parmar University of Horticulture and Forestry Solan, and three partners from the industry namely Ozone Biotech Limited Faridabad, Balaji Crop Care Pvt Limited Hyderabad, and Agri Food Park Ltd Pune.

The project has laid a sound foundation for research and development of cost effective technology and its transfer to industry. Over the period of time (Feb. 2009- Dec. 2012), a considerable progress has been made in developing technology for the production of anthocyanin concentrates from black carrot and jamun, lycopene concentrate from tomato, capsanthin concentrate from paprika, Steviol glycosides from *Stevia rebaudiana* and phycocyanin concentrate from *Spirulina*. Efforts have also been made to develop functional foods enriched with such nutraceuticals.

Successful implementation of the project has been made possible with the constant inspiration and encouragement by Dr Mruthyunjaya, Dr Bangali Baboo, and Dr D. Rama Rao National Directors (NAIP), Dr H. S Gupta, Director, IARI (Consortium Leader), Dr Malavika Dadlani, Joint Director (Chairperson, CIC), and Drs J.P. Mittal, R. K. Goyal and Dr R. Ezekiel, National Coordinators (component II). The guidance provided by Consortium Advisory Committee comprising of Dr Shiva Kumar, Former Director, National Institute of Nutrition, Hyderabad (Chairman), Dr D. S. Khurdia, Former Head, Division of Post Harvest Technology (Member), Prof Amir Azam, Department of Chemistry, Jamia Milia Islamia (Member), and Dr S. M. Gupta, Ranbaxy Laboratories, Gurgaon (Member) is gratefully acknowledged. My special thanks to Dr. Romesh Chand Rana, and Dr Ravinder Raina from Dr YS PUH&F Solan, Mr R.L. Narayan Rao, Managing Director Balaji Crop Care, Mr Pradeep Chordia, Managing Director, Agri Food Park Limited, Pune; and Mr Dhruv Gupta and Mr Venkat Gupta, Managing Directors, Ozone Biotech Limited Faridabad for their contribution. I wish to extend my appreciation and thanks to all the Co-PIs of the project namely Dr Pritam Kalia, Principal Scientist and Head, Vegetable Sciences, Dr Dolly Wattal Dhar and Dr Sunil Pabbi, Principal Scientists Microbiology; Dr Charanjit Kaur, Principal Scientist, Post Harvest Technology; Dr Jitendra Kumar and Dr N.A. Shakil, Principal Scientists and Dr Supradip Saha, Senior Scientist from the Division of Agricultural Chemicals for their valuable contribution and support in successful implementation of the project..The project team is also thankful to Heads, Divisions of Agricultural Chemicals, Division of Microbiology and Division of Post Harvest Technology, IARI, for providing necessary facilities. The help rendered by RAs and SRFs engaged in the project from time to time is also gratefully acknowledged.

Suresh Walia
Consortium Principal Investigator (CPI)

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Jan 27, 2014

Sub: Statement from CAC Chairman, Dr. B. Sivakumar on the NAIP Subproject No 50-56, component 2.

The NAIP sub-project on “**Value chain on production of food-grade nutraceuticals for use as natural antioxidants and food colorants**”, aimed at developing technical know-how for processes/products and protocols for efficient extraction, processing, purification, analysis, quality control, and formulation of food grade nutraceuticals and functional foods from vegetables, fruits, and non-food crops such as black carrot, tomato, chilli, jamun, *Stevia* and *Spirulina* was carried out by Dr Suresh Walia, Principal Scientist at Division of Agricultural Chemicals, Indian Agricultural Research Institute, New Delhi and other Consortium partners and collaborating Institutions during the years 2009-2012.

The project started with the baseline survey of sixty five farmers across four States to know the status of the nutraceuticals production and use. Under this sub-project, a number of processes, products and technologies have been developed and the progress of the project was monitored closely through CAC and CIC meetings. The technologies developed during the project implementation period include i) Extraction and purification of anthocyanins from black carrot and Jamun; ii). Production of capsaicinoids and capsanthin concentrates from Red Chilli (*Capsicum annum* L.); iii) Extraction technique of lycopene concentrate from tomato; iv) Production of phycocyanin concentrate from *Spirulina*; and v). Production of natural sweetener rich in stevioside and rebaudioside from *Stevia rebaudiana*. These technologies focused on low cost extraction protocol of the respective molecule(s) from the matrices, their characterization and content analysis and formulation of functional recipes. These nutraceutical molecules were functionalized into a value added foods such as juices, candy, ice-cream and bakery products. For example i) red guava nectar, ii) ready-to-serve, antioxidant rich mango juice and mango pana; iii) novel red amla candy; iv) a salsa formulation comprising of red capsicum and red tomato phytochemicals; and v) enriched bakery products such as golden/purple bread, biscuits, cakes, muffins,

as well as frozen products such as ice creams. Under this sub-project, novel micro and nano-sized nutraceutical formulations have been developed which include amphiphilic polymer based nano-formulation of β -carotene for slow release, phytosomal and inclusion complexes of black carrot/ jamun anthocyanins. Some of the technologies have been protected under IPR for their commercial use.

Success of the project can be further vouched from the fact that technologies developed under the project have been transferred to two industries for commercialization. The host institute (IARI New Delhi) has entered into an MOU with i) Ozone Biotech Faridabad, and ii) M/s Pratistha Industries, Secunderabad at a total consideration of 15.5 lakh. The sub-project yielded 3 patents, 5 research papers in international journals and two best poster awards. The NAIP sub-project also helped six persons to acquire advanced training and four scientists got international exposure in three different US universities. All the stakeholders and Consortium partners formed a strong cohesive network not only for success of the project, but also for future collaborations. The overall performance of the project has been excellent. This is one project where the deliverables were complete and all the objectives of the project have been met within reasonable time and budgetary support. The active participation of the CAC members and cooperation of the Consortium partners in shaping the project during execution is highly appreciated.


(Dr. B. Sivakumar)
Chairman, CAC

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सारांश

न्यूट्रास्यूटिकलस औषधीय गुणों से भरपूर होते हैं एवं सेहत को ठीक करने, जैसे रोगों को रोकने एवं उनके उपचार आदि में लाभ पहुंचाते हैं। कुदरती रंग भोजन को आकर्षक बनाकर उत्पाद में मूल्यवर्धन करते हैं। ऐसे भोज्य जिनमें मूल्यवान न्यूट्रास्यूटिकलस व रंग डाले जाते हैं उन्हें कार्यात्मक भोज्य, फर्टिफाइड भोज्य और या डिजाइनर भोज्य कहते हैं। उपभोक्ताओं का कार्यात्मक भोज्यों के प्रति बढ़ते झुकाव के कारण ऐसे भोज्य जो कुदरती स्रोतों से मिलते हों, की मांग में काफी बढ़ोतरी हुई है। न्यूट्रास्यूटिकलस अन्तर्राष्ट्रीय बाजार अभी भी बढ़ रहा है और इस समय इस उद्योग का ग्लोबीय आकार 125 विलियन से ऊपर है। इस समय भारत में न्यूट्रास्यूटिकलस का बाजार अपने बचपन में है और अन्तर्राष्ट्रीय बाजार में इसके हिस्सेदारी मात्र 1 प्रतिशत ही है। भारी क्षमता होने के नाते, भारतीय कृषि अनुसंधान परिषद, नई दिल्ली ने 'न्यूट्रास्यूटिकलस' को 'निसे' क्षेत्र में चिह्नित कर वर्ल्ड बैंक की वित्तीय सहायता से 'खाद्य श्रेणी के न्यूट्रास्यूटिकलस के उत्पादन एवं उनके कुदरती प्रतिआक्सीकारक एवं खाद्य रंग के उपयोग हेतु मूल्य चेन' नामक परियोजना (कोड 50:36) राष्ट्रीय कृषि नवोन्मेषी परियोजना के अन्तर्गत भारतीय कृषि अनुसंधान सस्थान, नई दिल्ली हेतु स्वीकृति 2009 में प्रदान की। इस परियोजना का मुख्य उद्देश्य उत्पाद एवं विधियों के बारे में तकनीकी ज्ञान तैयार करने के अतिरिक्त रंगों के निष्कर्षण, शोधन, गुणमान, गुणवत्ता नियंत्रण के साथ-साथ सब्जियों, फसलों व नॉन-खाद्य फसलों जैसे काली गाजर, टमाटर, मिर्च, जामुन, स्टीबिया व स्पाईरुलीना आदि से खाद्य श्रेणी के न्यूट्रास्यूटिकलस व क्रियात्मक भोज्य के विभिन्न संरूपण (उत्पाद) भी तैयार करना है।

चयनित सब्जियों एवं नॉन-भोज्य फसलों से प्राप्त खाद्य ग्रेड न्यूट्रास्यूटिकलस के वर्तमान उत्पादन एवं उपयोग को जानने हेतु एक आधारीय सर्वेक्षण किया गया। सर्वेक्षण हेतु हिमाचल प्रदेश, हरियाणा, उत्तर प्रदेश एवं आन्ध्र प्रदेश के पांच जिलों से 65 किसानों का चयन किया गया। चयनित किसानों से न्यूट्रास्यूटिकलस के बारे में आधारीय जानकारी प्राप्त करने के लिए एक विशेष प्रकार का फार्म तैयार किया गया जिसमें कई प्रकार के प्रश्न रखे गए। इस सर्वेक्षण से पता चला कि लोगों को सब्जियों, फलों या नॉन-खाद्य फसलों से प्राप्त होने वाले महत्वपूर्ण न्यूट्रास्यूटिकलस की जानकारी नहीं है। हालांकि किसान इन फसलों को केवल स्थानीय बाजारों में खपत की पूर्ति हेतु उगा रहे हैं। लेकिन इस परियोजना के बाद लोगों में न्यूट्रास्यूटिकलस-समृद्ध फलों और सब्जियों को उद्योगों में कच्चे माल के रूप में देने हेतु उत्सुकता बढ़ी। अतः हम चाहते हैं कि ऐसे फल व सब्जियों का उत्पादन बढ़ाया जाए ताकि इनकी घरेलू एवं अन्तर्राष्ट्रीय मांग पूरी की जा सके। इस राष्ट्रीय कृषि नवोन्मेषी परियोजना के अन्तर्गत निम्नलिखित विधियां, उत्पाद एवं तकनीकें विकसित की गईं। इनमें से कुछ उत्पादों का तकनीकी ज्ञान कुछ उद्योगों को भी स्थानांतरित किया गया है।

जामुन व काली गाजर से तैयार किया एंथोसाइनिन सांद्र

काली गाजर में कुदरती व स्थाई एसिलेटिड एंथोसाइनिन भारी मात्रा में पाया जाता है जो इसे गहरी जामुनी रंग प्रदान करता है। जामुन भी एंथोसाइनिन का अच्छा स्रोत है। काली गाजर एवं जामुन जूस, गूदे एवं सांद्र से एंथोसाइनिन को निकालने एवं इनके शोधन एवं विप्लेषण हेतु मूलभूत तकनीकी ज्ञान विकसित किया गया है। काली गाजर एवं जामुन के एंथोसाइनिन का कुदरती भोज्य रंग के रूप में उपयोग की अपार संभावनाएं हैं। काली गाजर का रूढ़िवादी या सीधे दाब की विधि द्वारा जूस हेतु प्रसंस्करण काफी कठिन तो है ही साथ में इससे जूस की मात्रा कम होती है और अवशेषों में काफी मात्रा में एंथोसाइनिन चले जाते हैं जो व्यर्थ ही जाते हैं। लेकिन जो निस्तारण मात्रा में तकनीकी हमने विकसित की है उसमें गाजर को ऊष्मीय उपचार के बाद कोषिका झिल्ली को निम्नकारी एंजाइम से भी उपचार किया जाता है ताकि जूस में एंथोसाइनिन की मात्रा अधिक हो। एंथोसाइनिन पाउडर (चूर्ण) सांद्र हेतु एवं विधि भी विकसित की है, जिसमें (i) काली गाजर एवं/या जामुन के गूदे से अर्क तैयार करना, (ii) अर्क को छानना एवं इसे मनचाहे अवशेषक से गुजारना, (iii) अवशेषक से निक्षालित रंग को किसी घोलक द्वारा निकालना एवं रंग (iv) एंथोसाइनिक का सांद्र प्राप्त करने हेतु रंग को सांद्र करना आदि का मानकीकरण किया गया। काली गाजर व जामुन के एंथोसाइनिन में एंथोसाइनिन के 3 से 4 घटक होते हैं। इनका एच.पी.एल.सी. (HPLC) से विप्लेषण किया गया एवं इनकी 'मास स्पेक्ट्रोस्कोपी' द्वारा संरचना ठीक पाई गई। एंथोसाइनिन सांद्र तैयार करने की विधि साधारण एवं लागत प्रभावी है। ऐसे एंथोसाइनिन स्वास्थ्य हेतु अतिलाभदायक होते हैं क्योंकि इनमें काफी मात्रा में प्रतिआक्सीकारक होते हैं एवं इनमें रोधक क्षमता बढ़ाने की दक्षता होती है। इनका प्रयोग न्यूट्रास्यूटिकलस-समृद्ध प्रसंस्कृत भोज्य, फल पेय, सुरा, जैम, कैंडी एवं हिमीकृत भोज्य जैसे आईसक्रीम, योमर्ट, ब्रैड, केक, सॉस, आहारी-संपूरक, प्रोबायोटिक्स आदि में आकर्षक बैंगनी

रंग व पोषकीय गुणवत्ता में सुधार हेतु किया जा सकता है। एंथोसाइनिन-समृद्ध काली गाजर की किस्में सब्जी विज्ञान संभाग, भारतीय कृषि अनुसंधान संस्थान, नई दिल्ली द्वारा विकसित की गई हैं, जिनकी खेती कर किसान उद्योगों को कच्चे माल के रूप में दे सकते हैं।

काली गाजर एवं जामुन के एंथोसाइनिन का जल में शीघ्र घुलना एक बहुत बड़ी समस्या है क्योंकि इनका कुछ हिस्सा ही मानव या जानवर के शरीर में जाता है और काफी हिस्सा बिना उपयोग हुए उत्सर्जित हो जाता है। इस समस्या के समाधान हेतु 'फाइटो कोमल संकुलन' को फोटोलिपिड्स सहित प्रयोग किया गया। ये फाइटोकोमल संकुलन मध्यम स्तर पर लिपिड में घुलते हैं जिससे एंथोसाइनिन की उपलब्धता मानव व पशुओं में बढ़ जाती है। एक दूसरे अध्ययन में बीटा-कैरोटीन, जो संतरी गाजर का मुख्य रंग रंगक है, को उभयस्नेही पॉलीमरिक मेट्रिक्स के साथ केप्सूलिकृत किया गया। ऐसे 'नेनो-आमापी' उत्पाद मध्यम स्तर से जल में घुलनशील होते हैं एवं काफी समय तक उपलब्ध होते हैं।

लाल मिर्च (केप्सीकम एनम एल.) से कैप्साइसीनाएड्स एवं केप्सेंथिन सांद्र

मिर्च, सोलानेसी कुल के कैप्सीकम वंश के सूखे लाल फल होती है। इस वंश की दो जातियां जैसे कैप्सीकम एनम एल. व कैप्सीकम फ्रूटेंसेस एल. ही आर्थिक महत्व की हैं जो ग्राम्य हैं। मिर्चों का लाल रंग केप्सेंथिन कैरोटिनायड वर्णक व तीखापन संबंधित कैप्साइसीनाएड्स जिसे कैप्साइसिन एवं डाइहाइड्रोकेप्साइसिन के कारण होता है, जो लगभग 90 प्रतिशत तीखापन हेतु उत्तरदायी होते हैं। जहां केप्सेंथिन को सर्वोत्तम तौर पर कार्यात्मक भोज्यों में रंजक के रूप में प्रयोग किया जाता है वहीं कैप्साइसीनाएड्स का प्रयोग उन क्रीमों में किया जाता है जो खारिष, मांसपेशियों के दर्द, जोड़ों के दर्द, पीठ के दर्द, टांगों के दर्द के निवारण हेतु बनी होती हैं। अतः 'पेपरिका' से कुदरती रंजक केप्सेंथिन एवं लाल तीखी मिर्च से सुरुचि कर्मक को निष्कर्षित करने की सुधरी विधि विकसित की गई है। यह एक साधारण एवं लागत प्रभावी विधि है। मिर्च में कैप्साइसीनाएड्स, केप्सेंथिन, टोकोफिरॉल एवं स्टीरॉल की मात्रा जानने हेतु जी.सी. एम.एस. एवं एल.सी.एम.एस. प्रोटोकॉल मानकीकृत कर लिए गए हैं।

टमाटर से लाइकोपीन सांद्र

टमाटर (लाइकोपरसीकोन एसकुलेंटस मिल्ल) कुदरती प्रतिऑक्सीकारक एवं रंजक जैसे 'लाइकोपीन' का बहुत अच्छा स्रोत है। लाइकोपीन, जो रंग घुलित लाल कैरोटिनाएड वर्णक है, की अधिक प्रतिऑक्सीकारक क्षमता के कारण इस तरह के यौगिक हेतु मांग बढ़ी है। यह यौगिक कैंसर, हड्डियों के कैल्सीकरण, आंखों का क्षय एवं तांत्रिक प्रणाली की क्षति आदि को रोकने में सहायता करता है। अतः छोटे स्तर पर ताजे टमाटरों एवं टमाटर के पेस्ट से लाइकोपीन सांद्र निष्कर्षण की तकनीकी जानकारी विकसित की गई है। लाइकोपीन-समृद्ध टमाटर की किस्में भारतीय कृषि अनुसंधान संस्थान, नई दिल्ली द्वारा विकसित की हैं जिनका उत्पादन कर किसान उद्योगों हेतु कच्चा माल की आपूर्ति कर सकते हैं। टमाटर की विभिन्न किस्मों, सांद्र एवं तकनीकी सामग्री से लाइकोपीन की सही मात्रा व विप्लेषण हेतु प्रोटोकॉल का मानकीकरण भी कर लिया गया है।

स्पाइरूलीना से फाइकोसायनिन सांद्र

स्पाइरूलीना को सम्पूर्ण कार्बनिक भोज्य माना जाता है क्योंकि इसमें पोषक तत्व जैसे विटामिन, खनिज, लिनोलेनिक अम्ल एवं कुदरती वर्णक जैसे कैरोटीन, क्लोरोफिल एवं फाइकोविलिन्स (फाइकोसायनिक, एलोफाइकोसायनिन एवं फाइकोएरीथ्रिन) आदि का समृद्ध स्रोत है। स्पाइरूलीना के विभिन्न प्रभेदों में वर्णक की सांद्रता भिन्न-भिन्न होती है। स्पाइरूलीना के तुलनात्मक अध्ययन से सी.सी.सी.-540, पी. एवं ए., सी.सी.सी.-480, सी.सी.सी.-482 आदि स्पाइरूलीना के प्रभेदों की पहचान हुई है। स्पाइरूलीना से अधिकाधिक वर्णक के उत्पादन हेतु वातावरण के विभिन्न परिवर्तकों का उपयोग कर प्रोटोकॉल मानकीकृत किया है। कार्बन डाईऑक्साइड, साधारण नमक एवं पी.एच. का फाइकोविलिन्स की सांद्रता के उत्पादन पर अनुकूल प्रभाव देखा गया है। मानकीकृत प्रोटोकॉल द्वारा स्पाइरूलीना की बायोमात्रा से फाइकोसायनिन का निष्कर्षण व शोधन किया गया। परिणामों से पता चला कि स्पाइरूलीना के फाइकोसायनिन समावयवी में एल्फा व बीटा 34-एकक (Sub-unit) पाए जाते हैं।

शोधकृत फाइकोसायनिन में SDS PAGE निष्कर्षण से एल्फा व बीटा 34-एककों को होने को संपुष्टि हुई। पी.सी.आर. (PCR) एंग्लीफिगेशन से पता चला कि फाइकोसायनिन के संश्लेषण हेतु cPcB-195-cPcA जीन जिम्मेवार हैं। स्पाइरूलीना के विभेदों का बीटा-कैरोटीन के आंकलन हेतु विप्लेषण भी किया गया है। स्पाइरूलीना की बायोमात्रा से फाइकोसायनिन के प्रभावी निष्कर्षण, शोधन एवं

एच.पी.एल.सी. (HPLC) विप्लेषण हेतु तकनीकी विधि विकसित की गई है। नीले वर्णक के निष्कर्षण के बाद, स्पाईरुलीना की बची बायोमात्रा का उपयोग सही ढंग से प्रोटीन संपूरक तैयार करने में किया जा सकता है। यह एक साधारण एवं लागत प्रभावी विधि है जिसमें किसी भी कार्बनिक घोलक का प्रयोग नहीं किया जाता है। स्पाईरुलीना से प्राप्त वर्णक एक बेहद कीमती है जिसका प्रयोग कुदरती प्रतिऑक्सीकारक एवं कार्यात्मक भोज्यों में आकर्षक रंग व संवेदी मान हेतु किया जा सकता है।

स्टीवियो रिवाओडियाना से स्टीवियोसाइड एवं रिबाओडियोसाइड समृद्ध मधुरक

स्टीविया रिवाओडियाना एक कुदरती मधुरक है जो चीनी से 250–300 गुणा मीठा है। मधुमेय के रोगियों द्वारा कुदरती मधुरक के रूप में स्टीवियोल ग्लाइकोसाइड्स का प्रयोग संसार में दिनोंदिन बढ़ रहा है। इन मधुरकों में मुख्यतः स्टीवियोसाइड्स व रिबाओडियोसाइड्स होते हैं। स्टीविया से स्टीवियोल ग्लाइकोसाइड निष्कर्षण की तकनीकी विधि विकसित की गई है। यह विधि लागत-प्रभावी, साधारण एवं दक्ष है। मधुरकों को आयन विनिमय, रेजिन, एवं क्रोमेटोग्राफी द्वारा शोधित किया गया है। स्टीवियोल के पृथक्करण के अतिरिक्त, उत्पाद में कड़वापन कम करने हेतु भी कोषिष की गई। स्टीविया के अर्क एवं सांद्र से स्टीवियोसाइड्स व रिबाओडियोसाइड्स के विप्लेषण एच.पी.एल.सी (HPLC) विधि मानकीकृत की गई है।

न्यूट्रास्यूटिकल-समृद्ध कार्यात्मक भोज्य

बजार में अमरुद के पेय सफेद व लाल रंगों में मिलते हैं। ऐसे लाल पेयों में सफ़लेषी रंग (ई-224) का प्रयोग किया जाता है जो मनुष्य के लिए हानिकारक हो सकता है। सेहत के प्रति बढ़ती जागरूकता के कारण, उपभोक्ताओं का झुकाव सेहत के लिए सुरक्षित रसायनों की ओर तेजी से बढ़ रहा है। अतः अमरुद का लाल नेक्टर एवं त्वरित उपयोग हेतु पेय (आर.टी.एस.) तैयार किया गया है जिसमें कुदरती एसिलेटिड एंथोसाइनिन होते हैं जो पेय को गुलाबी रंग एवं स्वास्थ्यवर्धक बनाता है। आम पर आधारित त्वरित पेय जैसे आम का ड्रिंक व आम का पन्ना बाजार में मिलते हैं। नवीन विकसित किए गए 'न्यूट्रा-मैंगो-पन्ना' में कुदरती एसिलेटिड एंथोसाइनिन हैं जिसे ई-सूचीकरण की कोई आवश्यकता नहीं है। उत्पाद में व्याप्त 50–100 मि.ग्राम/ली. एंथोसाइनिन उत्पाद को स्थाईपन प्रदान करते हैं। इसी तरह 'नवीन आंवला कैन्डी' में आंवले व काली गाजर के क्रियात्मक अवयवों का संगम है। जबकि चीनी के सीरप में आंवले के कुदरती लाल रंग में समृद्ध टुकड़ों में एंथोसाइनिन लगभग 9 महीनों तक बना रहता है। सूखी कैन्डी को 'डिस तकनीक' से बनाया गया है जिसे निर्वात में पैक करने से साधारण तापमान पर 6 महीने तक सुरक्षित रखा जा सकता है। इसके अतिरिक्त कुदरती एंथोसाइनिन आंवले के उत्पादों को आकर्षक दिखावट व संवेदीमान प्रदान करते हैं। मौसम-विषेय में अधिक उत्पादन से टमाटर व भिमला मिर्च का अधिकाय हो जाता है, जिससे बाजार में इनके भाव काफी गिर जाते हैं एवं किसानों को कई बार बहुत क्षति उठानी पड़ती है। अतः लाल भिमला मिर्च एवं लाल टमाटरों से 'सालसा' तैयार किया गया है, जिसमें दोनों टमाटर व भिमला मिर्च के लाभ व्याप्त हैं। ताजे लाल मिर्च व टमाटरों को प्रसंस्कृत कर घनी सौस जैसा उत्पाद-सालसा तैयार किया गया है जिसमें मसाले, सुस्वाद एवं अन्य अवयव डाले गए हैं। सालसा बनाने हेतु टमाटर की प्यूरी को मनचाही घनेपन तक पकाया गया एवं अन्तिम उत्पाद को कुदरती परिरक्षक परिरक्षित द्वारा किया गया। न्यूट्रास्यूटिकल-समृद्ध बेकरी उत्पाद जैसे गोल्डन व बैंगनी ब्रेड, बिस्कुट, केक, मफिनस एवं हिमीकृत उत्पाद जैसे आईस्क्रीम को तैयार करने हेतु तकनीकी विकसित की गई है।

इस परियोजना की सफलता का पता इन बातों से भी चलता है कि जो तकनीकें इस परियोजना के अन्तर्गत विकसित की गई हैं उनका हस्तान्तरण दो उद्योगों को किया गया है। भारतीय कृषि अनुसंधान संस्थान, नई दिल्ली ने मै. ओजोन बायोटेक, फरीदाबाद एवं मै. प्रतिष्ठा इंडस्ट्रीज, सिकन्दराबाद से 15.5 लाख रुपये में समझौता ज्ञापन पर हस्ताक्षर किए हैं। ये दोनों उद्योग न्यूट्रास्यूटिकल-समृद्ध उत्पादों के व्यावसायिक उत्पादन हेतु फैक्टरी लगाने जा रहे हैं।

EXECUTIVE SUMMARY

Nutraceuticals provide medical and health benefits, including the prevention and treatment of a disease. Natural colorants make food more attractive and add value to the products. Foods incorporated with high value nutraceuticals and food colorants are commonly referred to as functional foods, fortified foods, and/or designer foods. The increasing interest of consumers in nutraceuticals and functional foods with health capabilities has brought about a rise in demand for functional ingredients from natural sources. The international market for nutraceuticals is still expanding and at present the global size of the industry is over 125 billion. At present Indian nutraceutical market is in its infant stage having less than 1% share of the international market. With increasing awareness about health and the risk of lifestyle disorders, consumers are looking at nutraceuticals as effective products.

In view of the huge potential, ICAR identified “Nutraceuticals” as a niche area of research and sanctioned a World bank funded NAIP project on “Value chain for the production of food grade nutraceuticals for use as natural antioxidants and food colorants” (Code 50:56) to IARI, New Delhi in 2009. The project aimed at developing technical know-how for processes/ products and protocols for efficient extraction, processing, purification, analysis, quality control, and formulation of food grade nutraceuticals and functional foods from vegetables, fruits, and non-food crops such as black carrot, tomato, chilli, jamun, *Stevia* and *Spirulina*.

At the onset of the project, baseline survey was conducted to know the present status of production and use of food grade nutraceuticals from selected vegetables, fruits and non-food crops. Sixty five farmers spread over five districts of Himachal Pradesh, Haryana, Uttar Pradesh, and Andhra Pradesh, were selected to prepare the baseline report. A set of questionnaire was used to collect desired information from the respondents at village and farmers levels. Baseline survey has indicated that at present farmers are unaware of nutraceutical potential of vegetables, fruits and non-food crops. They grow these crops to meet local consumption requirements only. After the project intervention farmers were eager to grow nutraceutical rich vegetable and fruit crops for use as raw material by the industry. We therefore, need to augment all resources at our command to increase the production of nutraceuticals and functional foods to meet both domestic and international demand. Under this NAIP sub-project following processes, products and technologies have been developed. Technical know-how of some of the products has been transferred to industries.

All the proposed work was executed according to the plan mentioned in the project document.

❖ **Anthocyanin concentrate from black carrot and Jamun**

Black carrot, a rich source of natural stable acylated anthocyanins which impart black carrots their dark violet color. Jamun is also a good source of anthocyanins. Bench scale know-how has been developed for the extraction, purification, analysis and quality control of anthocyanins from black carrot and jamun juice, pulp and/or concentrate. The developed extraction technology employs commercial hot breaking heat treatment followed by treatment with cell wall degrading enzyme(s) for recovering higher amounts of anthocyanins in the juice with lesser wastes. A method of making anthocyanin powder concentrate has been developed which includes (a) extraction of pulp, ii) filtering and passing the extract through a adsorbent column, iii) eluting the colorant with a suitable solvent, and iv) concentrating the elute to provide anthocyanins concentrate. The black carrot and jamun concentrates contained 3 to 4 major

anthocyanin constituents. These concentrates were analyzed for active ingredients by HPLC and their structures were confirmed by mass spectroscopy. The process of anthocyanin concentrate production is simple and cost effective. The anthocyanin rich black carrot varieties developed in the Division of Vegetable Sciences at IARI can be taken up for cultivation by the farmers to provide raw material to the industry.

❖ **Capsaicinoids and capsanthin concentrates from Red Chilli (*Capsicum annum* L.)**

Chilies are the dried red fruits of the genus *Capsicum* and family Solanaceae. The two well known species, *Capsicum annum* L. and *C. frutescens* L. are economically important of the domesticated chilli peppers. The red color of chilies is due to carotenoid pigment like capsanthin and the hot flavour is due to a group of closely related capsaicinoids namely capsaicin and dihydrocapsaicin that account for approximately 90% of the pungency. An improved process has been developed for extraction of food grade natural colorant capsanthin from paprika and a flavouring agent capsaicinoids from red hot chilli. The process is simple and cost effective. GC/MS and LC-MS protocols have been standardized for quantitation of the capsaicinoids, capsanthin, tocopherol, and sterol content in chili.

❖ **Lycopene concentrate from tomato**

Tomato is a rich source of natural antioxidant and colorant like lycopene. High antioxidant activity of lycopene, a fat soluble red carotenoid pigment has increased the demand of this group of compounds. Bench scale know-how has been developed for the extraction of lycopene concentrate from fresh tomato and/or tomato paste. The process is simple, cost effective and efficient. Varieties rich in lycopene content have been developed in the Division of Vegetables at IARI which can be recommended to farmers who wish to cultivate tomato crop as raw material to the industry. Protocols have also been standardized for accurate analysis and quantitation of lycopene in different tomato varieties, concentrates and technical materials.

❖ **Phycocyanin concentrate from *Spirulina***

Spirulina is considered as a complete organic food source enriched with nutrients like vitamins, minerals, linolenic acid, and natural pigments like carotenes, chlorophyll, and phycobilins (phycocyanin, allophycocyanin and phycoerythrin). The blue pigment concentration varied distinctly amongst different strains. Comparative studies of *Spirulina* led to identification of *Spirulina* strains (CCC 540, P&A, CCC 480, CCC 482) in terms of growth potential and pigment profile. The growth of *Spirulina* sp. (CCC540) was also compared in photobioreactor system *vis a vis* stirred raceways lodged in a polyhouse. Protocol for enhanced production of pigments from *Spirulina* was standardized by varying environmental variables. Distinct increase was seen in the concentration of phycobilins with increase in CO₂, salt (NaCl) and/or pH effect. *Spirulina* biomass was subjected to extraction and purification of phycocyanin through optimized protocol. SDS PAGE analysis confirmed the presence of α and β subunit for purified phycocyanins. PCR amplification confirmed the presence of *cpcB-IGS-cpcA* gene responsible for phycocyanin synthesis. *Spirulina* strains have also analyzed for β -carotene estimation.

Technical know-how (process) has been developed for efficient extraction, purification and HPLC analysis of phycocyanins from *Spirulina* biomass. After extraction of the blue pigment, the remaining *Spirulina* biomass can be appropriately formulated and used as protein supplement. The method is cost effective, simple and does not involve use of undesired organic solvents. .

❖ **Stevioside and rebaudioside rich natural sweetener from *Stevia rebaudiana***

Stevia rebaudiana is a rich source of natural sweetener which is 250-300 times sweeter than sugar. Steviol glycosides are increasingly used world over as natural sweetener particularly by diabetic patients. These sweeteners comprised of mainly steviosides and rebaudiosides. Know-how has been developed for the extraction of steviol glycosides from *Stevia* sp. The process is simple, cost effective and efficient. The sweeteners were further purified with ion exchange resins and/or column chromatography. Besides separation of steviol glycosides, attempts have also been made to reduce bitterness of the product. HPLC method has been standardized for simultaneous analysis of steviosides and rebaudiosides in the stevia extracts and concentrates.

❖ **Novel micro and nano-sized nutraceutical formulations with increased efficacy**

Ready solubility of black carrot and jamun anthocyanins in water is a bottleneck as only a fraction of anthocyanin ingested is taken up by the human/animal system. A significant part remained un-utilized and excreted out. Naturally occurring phospholipids having water soluble hydrophilic head and lipid soluble hydrophobic tail, have been used to develop phytosomal complexes of black carrot and/or jamun anthocyanins. Such nano-sized micelles of phytosomal complexes are more bioavailable and exhibit enhanced health benefitting effects. Cyclodextrin inclusion complexes of anthocyanins have also been developed that have homogeneous plate like structures (SEM) and are thus more effective. Efforts have been made to develop amphiphilic polymer based nano-formulation of β -carotene for slow release as well as higher bioavailability and stability. Due to its nano size, the encapsulated β -carotene is more effective and showed extended release time. Red chilli (paprika) derived capsanthin, being lipophilic has limited water solubility and reduced bioavailability. Encapsulation of capsanthin oleoresin with dextrin and PEG based polymers has resulted in a standard recipe which may find application in food industry. Spirulina derived blue pigment- phycocyanin is highly soluble in water. Encapsulated products of phycocyanin following complexation with maltodextrin, guar gum, gum arabic, and /or chitosan have been developed to regulate its solubility in water. Such novel micro and nano-sized nutraceutical formulations of anthocyanins, β -carotene, capsanthin, and phycocyanin, being more effective will find wider application in food industry.

❖ **Nutraceutical-enriched functional foods**

Guava beverages available in the market as white and red variants are usually colored with synthetic colorants (E-224) which can be harmful to humans. Red guava nectar, a ready-to-serve (RTS) drink containing natural acylated anthocyanins exhibit characteristics pink color and imparts health promoting benefits to the drink. Mango based ready-to- serve drinks like mango juice and mango pana are usually available in natural variants. The newly developed nutri-mango pana contains natural acylated anthocyanins which does not require E-labelling. Anthocyanin content ranging between 50-100mg/L imparts high stability to the products. Similarly novel red amla candy combines the biologically active components from amla and black carrots. While red amla pieces in syrup enriched with natural colorant has high stability for anthocyanins for 9 months at low temperature, dried candy processed through DIS process, and vacuum packed have good shelf life for 6 months. Further natural anthocyanins provide a pleasing sensory appeal to the amla products.

During the peak season there is a huge glut of tomato and capsicum because of over production. A salsa formulation comprising of red capsicum and red tomato component has been developed which has the benefit of both tomato and capsicum antioxidants. Freshly harvested red capsicum and tomato were

processed to obtain a thick sauce like product - salsa containing spices, flavors and other ingredients. The puree was cooked to a desired consistency and final product preserved with natural preservatives. Know-how has also been developed for the preparation of nutraceutical enriched bakery products such as golden/purple bread, biscuits, cakes, muffins, as well as frozen products such as ice creams.

❖ **Transfer of technology to industry**

Success of the project can be further vouched from the fact that technologies developed under the project have been transferred to two industries for commercialization. The host institute (IARI New Delhi) has entered into an MOU with the companies namely i) Ozone Biotech Faridabad, and ii) M/s Pratistha Industries, Secunderabad at a total consideration of 15.5 lakh (7.5 + 8.0 lakh, non-exclusive). These industries are in the process of setting up the plant for commercial production of high-value nutraceuticals and functional foods.

Significant achievements

- Production technologies developed and adopted : 9
- Process technologies developed, adopted and commercialized : 6
- Rural industries established/commercialized : -
- Patents (filed/granted) : 3
- Publications:
 - i. Research papers published : 7
 - ii. Popular articles published : -
 - iii. Books/book chapters published : 3
 - iv. Bulletins/brochures/leaflets published : 1
 - v. Training manuals published : 3
 - vi. Film/ CD developed : 1
 - vii. Coverage in press, TV, media : 1
- Trainings undertaken and scientists/other staff trained (national/international) : 6
- Trainings organized and farmers/other stake holders trained : 4
- Field demonstrations organized : 2
- Field day/farmer day/'mela' organized : 2
- Success stories : 2
- Self help groups/farmer groups developed :-
- Employment generation (man days/year) :-
- Assets generation (equipments/implements procured, civil work done and revenue generated)
 - : 44 equipments (3.28 crore) & 5 laboratory developed/renovated (31 lakhs), Revenue 15.5 lakhs generated
- Awards/honors : 2

Socio-Economic Impact: Developed technologies have been transferred to two industries and once the production of these nutraceuticals will start the value chain will be strengthened and its economic impact especially among farmers will be distinctly visible.

Environmental Impact: The project does not foresee any ecological and environmental degradation as it deals with products and technologies derived from vegetables, fruits and other non-food crops. Given the present status of nutraceuticals and food industry and the activities of the sub-project, no adverse effect on environmental and social safeguards is anticipated.

Sustainability Plan:

1. After completion of the project, all the consortium partner will continue to organize trainings/ demonstrations to potential stake holders/ industries.
2. Project scientists will continue to promote cultivation of potential nutraceutical rich vegetables varieties among farmers.
3. Efforts will be made to identify some more potential entrepreneurs for transfer of technologies which will ultimately benefit the farmers and the industry.
4. Scientists will continue to develop technical know-how for cost effective production of value added nutraceuticals from un-explored vegetables, fruits, microalgae, and marine produce for their nutraceutical potential.
5. Research infrastructure created under the project will be made available to other NARS scientists to enable them develop standard protocols for the analysis and quality control of nutraceuticals and functional foods to enhance the scope of domestic and international demand.

Part-I: General Information of Sub-project

1. **Title of the sub-project:** Value chain on production of food-grade nutraceuticals for use as natural antioxidant and food colorants
2. **Sub-project code:** 50-56
3. **Component:** 2
4. **Date of sanction of sub-project:** February 13, 2009
5. **Date of completion:** Dec. 30, 2012
6. **Extension if granted, from** July 1, 2012 to December 31, 2012
7. **Total sanctioned amount for the sub-project:** Rs: 636.8665 lakh
Total expenditure of the sub-project: Rs. 628.13824 lakh
8. **Consortium leader:**
Dr H. S. Gupta
 Director
 Indian Agricultural Research Institute
 New Delhi-110 012

9. **List of consortium partners:**

	Name of CPI/ CCPI with designation	Name of organization and address, phone & fax, email	Duration (From-To)	Budget (Lakhs)
CPI	Dr Suresh Walia, Principal Scientist	Division of Agricultural Chemicals, Indian Agricultural Research Institute New Delhi- 110 012, 011 25841390, 011-25843272 , suresh_walia@yahoo.com ,	2009-2012	563.8797
CCPI1	Dr. Ramesh Rana	Department of Forest Products, Y.S.Parmar Univ. of Hort.& Forestry, Solan, 09418458241, romeshchandrana@yahoo.co.in	2009-2012	46.43894
CCPI2	Mr Pradeep Chordia	Managing Director, Agri Food Park Ltd. Pune, 020-32903850, pradeep@chordia.com	2009-2012	6.36.35
CCPI 3	Mr Dhruv Gupta	Managing Director, Ozone Biotech Limited, Faridabad	2009-2012	10.2375
CCPI 4	Mr. R.L. Narayana Rao,	Managing Director, Balaji Crop Care Pvt Limited Hyderabad	2009-2012	9.95
Total				636.8665

CPI-Consortia Principal Investigator; CCPI-Consortia Co-Principal Investigator

10. Statement of budget released and utilization partner-wise (in Lakhs):

	CPI/ CCPI Name, designation & address)	Total budget sanctioned	Fund released (up to closing date)	Fund utilized (up to closing date)
CPI	Dr Suresh Walia, Division of Agricultural Chemicals, Indian Agricultural Research Institute, New Delhi-110 012	563.8797	563.8797	558.17081
CCPI 1	Dr. Ramesh Rana, Department of Forest Products, Y.S.Parmar Univ. of Hort.& Forestry, Solan	46.43894	46.43894	43.94450
CCPI 2	Mr Pradeep Chordia, Managing Director, Agri Food Park Ltd. Pune	6.36035	5.83535	5.83535
CCPI 3	Mr Dhruv Gupta, Managing Director, Ozone Biotech Limited, Faridabad	10.2375	10.2375	10.23750
CCPI 4	Mr. R.L. Narayana Rao, Managing Director, Balaji Crop Care Pvt Limited Hydrabad	9.95	9.95	9.95
Total		636.8665	636.34162	628.13824

CPI-Consortia Principal Investigator; CCPI-Consortia Co-Principal Investigator

Part-II: Technical Details

1. Introduction

Consumption of vegetables and fruits is associated with lower incidence of cardiovascular, cancer, obesity and other human diseases. The type of vegetables and fruits that most often appeared to be protective against these diseases includes green vegetables, chilli, carrots, beet root and tomatoes (Block et al 1992). Nutraceuticals derived from high value fruit and micro-algal extracts also find application as dietary supplements, food colorants and functional foods. Such products provide medical or health benefits, including the prevention and treatment of a disease. Dietary ingredients in a food supplement include a vitamin, a mineral, an herbal/ botanical product or concentrate, an amino acid, enzyme or tissues from organs or glands, a secondary metabolite constituent or extract. These can be given in many novel forms such as tablets, capsules, soft-gels, bars, liquids or powders. Natural colorants make food more attractive and add value to the products. Foods incorporated with high value nutraceuticals and food colorants are commonly referred to as functional foods, fortified foods, designer foods and/or processed foods such as cereals, soups, bread-spreads, dressings and beverages enriched with nutraceuticals. The increasing interest of consumers in functional foods with health capabilities has brought about a rise in demand for functional ingredients from natural sources.

Nutraceutical revolution began in the US, Europe and Japan in early 1980s and later spread all over the world. The international market for nutraceuticals is still expanding and at present the global size of the industry is over 125 billion. The projected rate of growth in the U.S. is about 15 per cent per annum. Usually obtained from a food source, these are sold as concentrates or purified components in pharmacological doses for specific ailments or used for the manufacture of functional foods. In India where increased population rate and poverty face the malnutrition problem, nutraceutical rich vegetables, fruits and carotenoid-rich indigenous microalgae appear to be boon. India's population today exceeds 1.25 billion, with a large number living in small rural villages. It is growing by 20 million annually and is becoming increasingly urban. With the second largest population in the world, India presents excellent opportunities for nutraceuticals and dietary supplements. Consumers are increasingly becoming health-conscious and are realizing the need for dietary supplements to help them cope with the fast-changing pace of life, which has put them at the risk of diabetes, cholesterol, heart ailments, arthritis etc. Most of the dietary supplements in the form of functional food are tasteless and many of them are not manufactured in India. Further most of the nutraceutical products available in high end stores generally lack basic details regarding composition dosage etc. on the label.

The nutraceutical market in India is currently estimated between Rs 15-20 billion and is far behind the European and US markets. At present the level of exports from India is less than Rs. 750 crores. India has, however, the huge potential of becoming a big player in this industry provided it develops scientific basis to support claims of safety, efficacy and quality. In view of the huge potential, it is proposed to develop know-how for the production of food grade nutraceuticals from vegetables like carrot, tomato and capsicum, fruits like jamun, non-food crop like *Stevia* and nutraceutical rich micro-algae. Nutraceuticals

so obtained from these materials will find wide application in the nutraceutical, pharmaceutical, cosmaceutical and health food industry.

2. Overall Sub-project Objectives

1. Protocol optimization for extraction and processing of food grade nutraceuticals and food colorants from vegetables, fruits, non-food crops and microalgae
2. Isolation, chemical characterization, analysis and evaluation of active ingredients for anti-oxidant and other properties
3. Development of analytical protocols for quantification of nutraceuticals in technical materials and finished products
4. Development of innovative nutraceutical formulations of with extended shelf life
5. Scale-up, transfer and sharing of know-how with industry and other stakeholders

3. Sub-project Technical Profile

(Indicate briefly objective-wise work plan, monitoring indicators, expected output and expected outcome)

S. No.	Project activity	Expected outcome	Measurement	When	Linkage monitoring by
Objective 1: Protocol optimization for extraction and processing of food grade nutraceuticals and food colorants from vegetables, fruits, non-food crops and microalgae					
1.	Cultivation of vegetable crops (carrots, tomato, and chilli) and procuring of jamun)	Nutraceutical rich vegetable varieties and fruits	Number of nutrition-rich vegetables and fruits	2009-2011	CMU/CAC/CIC
2.	Cultivation of the non-food crop <i>Stevia rebaudiana</i>	Sugar-free <i>Stevia</i> plantation	Stevia plantation in more area of Himachal Pradesh	2010-2012	CMU/CAC/CIC
3.	Develop and optimize protocols for extraction of food grade lycopenes, carotenes and anthocyanins from tomato, black carrots, pomegranate and jamun	Nutraceutical concentrates	Nutraceutical concentrates from number of vegetables and fruit like jamun	2009-2011	CMU/CAC/CIC

4.	Develop and optimize protocols for extraction of food grade capsanthin from chilli	Capsanthin-rich red chilli concentrates	Improvement in extraction technology	2009-2011	CMU/CAC/CIC
5.	Develop and optimize protocols for extraction of food grade steviosides from <i>Stevia rebaudiana</i>	Stevia concentrates	Quantity and quality of <i>Stevia</i> concentrates	2010-2012	CMU/CAC/CIC
6.	Develop and optimize protocols for production of microalgae and extraction of natural colorants from <i>Spirulina</i>	<i>Spirulina</i> biomass and concentrates	Quantity and quality of <i>Spirulina</i> biomass and blue pigment	2010-2012	CMU/CAC/CIC
7.	Develop and optimize protocols for isolation and purification of food grade phytochemicals from vegetables, <i>Stevia</i> and microalgae	Purified phytochemicals/ nutraceuticals	Standardized Purification protocols	2009-2011	CMU/CAC/CIC
Objective 2: Isolation, chemical characterization and evaluation of active ingredients for anti-oxidant and other properties					
1.	Process development and chemical characterization of antioxidants and food colorants from <i>Spirulina</i> spp	<i>Spirulina</i> biomass and food colorant	Quantity of biomass and blue pigment - phycocyanin	2009-2012	CMU/CAC/CIC
2.	Process development and chemical characterization of nutraceuticals (carotenes, lycopene, anthocyanins) from vegetables and fruits	Indigenous know-how and Standard nutraceuticals from vegetables and fruits	Purity level of nutraceuticals /food colorants from vegetables/ fruits	2009-2011	CMU/CAC/CIC

3.	Process development and chemical characterization of capsaicinoids and capsanthin pigment from chilli	Indigenous know-how and Standard nutraceuticals from chilli	Purity level of nutraceuticals /food colorants from chilli	2009-2011	CMU/CAC/CIC
4.	Process development and chemical characterization of steviol glycoside from <i>Stevia rebaudiana</i>	Indigenous know-how for standard natural steviol glycosides as sugar substitute	Purity level of sugar free steviosides and rebaudiosides	2009-2011	CMU/CAC/CIC
5.	Evaluation of nutraceuticals for their anti-oxidant, food colorant and other properties	Extent of antioxidant activity	Bioactivity level	2009-2011	CMU/CAC/CIC

Objective 3: Development of analytical protocols for quantification of nutraceuticals in technical materials and finished products

1.	Development of nutraceutical-rich fusion products and functional foods	Innovative Fusion food products	Quality assessment	2010-2012	CMU/CAC/CIC
2.	Development and standardization of analytical techniques (LC/ LC-MS) for identification and quantification of nutraceuticals in plant materials, technical concentrates and fortified functional foods.	Number of analytical methods (GC/GC-MS/LC/LC-MS etc) developed for analysis of nutraceuticals	Standard analytical protocols and parameters for accurate analysis	2010-2011	CMU/CAC/CIC
3.	Analysis of chlorophyll, carotenoids (carotenes	Number of analytical techniques	Standard Analytical protocols and	2010-2011	CMU/CAC/CIC

	and xanthophylls) and phycobilins in <i>Spirulina</i>		parameters		
4.	Analysis of steviol glycoside contents in <i>Stevia rebaudiana</i> extractives and technical materials	Number of analytical techniques	Standard Analytical protocols and parameters	2010-2011	CMU/CAC/CIC
Objective 4: Development of innovative nutraceutical formulations of with extended shelf life					
1.	Identification and use of food grade stabilizers to prevent degradation of nutraceutical ingredients in technical materials and finished products	Selected stabilizers	Stabilizing efficiency	2009-2011	CMU/CAC/CIC
2.	Develop innovative stable solid and liquid products/formulations of potential nutraceuticals	Stable formulation/ fusion products/ encapsulated materials/ phytosomal complexes etc	Novel fusion products/ formulations with enhanced efficacy	2009-2011	CMU/CAC/CIC
Objective 5: Scale-up, transfer and sharing of know-how with industry and other stakeholders					
1.	Scale up of technology for large scale production	Scaled up technical know-how	Level of scale up	2008-2010	CMU/CAC/CIC
2.	Transfer of technology to industry and other stakeholder	Technology transfer	Industry acceptance of technology	2010-2012	CMU/CAC/CIC
3.	Establish market linkage among the stakeholders to promote domestic and	Number of entrepreneurs entered into the venture	Acceptance by the entrepreneurs/ stakeholders	2010-2011	CMU/CAC/CIC

	export potential of nutraceuticals, food colorants and functional foods.				
4.	Sharing of information with stakeholders and final reports writing	New products and know-how	Successful commercialization	2011-2012	CMU/CAC/CIC

Expected output

- ☐ Bench scale know-how for the production of natural antioxidants such as carotenes, lycopenes and anthocyanins from tomato and black carrot
- ☐ Indigenous know-how for the production of the natural food colorants capsanthin and capsaicinoid concentrates from chilli.
- ☐ Indigenous know-how for the production of low-calorie steviol glycosides from *Stevia rebaudiana* for use as sugar free substitute
- ☐ Know-how for the production of natural food colorant and antioxidant from the micro-algae *Spirulina*
- ☐ Innovative products (oleoresins, pure and technical materials, beverages, sport bars fusion foods etc.) from vegetables and fruits for commercial use.
- ☐ Improvised QC and analytical protocols for analysis and quantification of nutraceuticals in technical materials and fusion foods

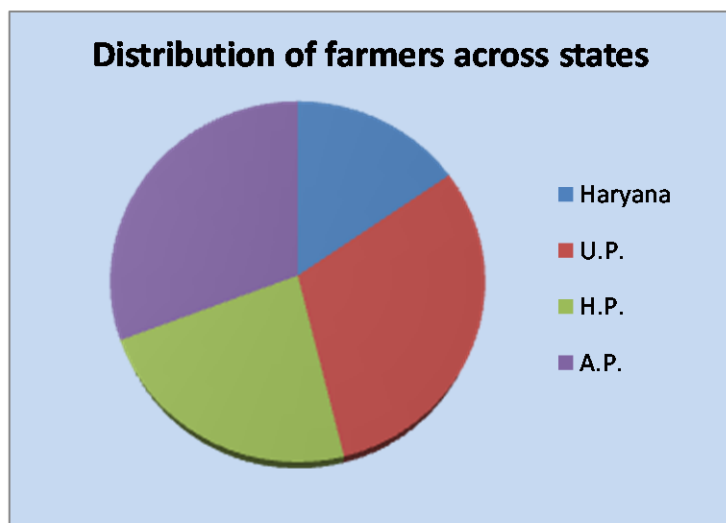
Expected Outcome

- ☐ Increased cultivation of the nutraceutical-rich vegetables, fruits and other non-food crops will result in increased income to the farmers
- ☐ Nutraceutical-rich vegetables and fruits may result in the replacement of the less remunerative crops
- ☐ Increased availability of vegetable, fruit and algal based antioxidants and food colorants
- ☐ Enhanced export of the standard quality nutraceuticals, food colorants and fusion products will increase the market share of Indian manufactured products in the international market
- ☐ Skilled human resource on value-added nutraceuticals and functional foods
- ☐ Increased linkage with the food processing industry
- ☐ Generation of intellectual property/ patents relating to new processes and products

4. Baseline Analysis

Baseline survey was conducted to know the present status of production and use of food grade nutraceuticals from selected vegetables, fruits and non-food crops. Sixty five farmers spread over five districts of Himachal Pradesh, Haryana, Uttar Pradesh, and Andhra Pradesh, were selected to prepare the baseline report. A set of questionnaire was used to collect desired information from the respondents at village and farmers levels. The significance and salient features of the project is briefly highlighted below. Baseline survey has indicated that at present farmers are unaware of nutraceutical potential of

vegetables, fruits and non-food crops. They grow these crops to meet local consumption requirements. Some multinational and domestic food processing industries like Hindustan Lever, ITC, Nestle India, Britannia Industries, Parry Agro, Heritage Foods, Agro-Dutch Industries, Agro-Tech, Rei Agro, Temptation Foods, Chordia Foods, and the likes are engaged in general food processing. Most of the companies have, however, not entered the value-added nutraceutical, antioxidant, natural food colorant,



and functional food business. In view of their nutraceutical attributes and health benefits, such products have great domestic and export potential. Through the NAIP sub-project intervention, select farmers in the select villages will be encouraged to produce nutraceutical rich vegetables/fruits so that these are available in plenty for consumption as raw material for the food processing/nutraceutical industry. As a component of value chain, efforts will be made to develop indigenous know-how for the production of natural antioxidants and food colorants from

vegetables, fruits and non-food crops. Indigenous know-how so developed will be made available to the food processing industry. In view of the benign nature and ease of production such products/ processes/ technologies shall have no adverse effects on the environmental and social safeguards. Besides generating income of the farmers and concerned stakeholders, such products will help provide nutritional security to our vast population.

Following conclusions were made based on the base line survey made in five districts across four states.

Farmer's practices

- ❖ Under present practices of vegetable cultivation, farmers are resorting to cultivation of conventional vegetables and fruits.
- ❖ Though, cultivation of vegetables and fruits is remunerative, yet, there is good scope of value addition in terms of producing natural antioxidant and food colorants from vegetables and fruits as a critical component of functional foods.
- ❖ Majority of the farmers do not know the significance of nutraceutical in food industry

Vegetable/fruit growers are of the opinion that proper training and inputs should be provided to the farmers to encourage them take up more area under cultivation. Farmers are not familiar with cooperatives or buy-back systems for marketing their produce (vegetables and fruits) by the industry.

Nutraceutical production

- ❖ At present, nutraceutical production from vegetables and fruits is non-significant
- ❖ Indigenous know-how for production of nutraceuticals such as natural antioxidants, natural food colorants, and functional food is not available.

- ❖ A large number of nutraceutical products such as carotene from carrot, lycopene from tomato, anthocyanins from black carrot and other fruits, polyphenols from pomegranate, phycobillins from Spirulina are available in the international market. However, none of these health products are indigenously produced in India.
- ❖ Innovative extraction and product development technologies needs to be developed to ensure availability of natural antioxidants and food colorants in India.
- ❖ Adequate research infrastructure must be developed for production/analysis & quality control of nutraceuticals and functional foods from vegetables/fruits/non-food crops.

Functional foods

- ❖ As compared to the developed countries like USA, Europe, Australia, Japan etc, where a large variety of functional foods enriched with nutraceuticals are available, availability of such products is very limited in India
- ❖ In view of the increasing demand for functional/fortified foods due to ever increasing life style diseases, the demand for such products is increasing.

NAIP sub-project in reference aims at developing indigenous technical know-how for production of much needed value-added nutraceuticals and functional foods for both domestic consumption and export.

Expected income generation

Raw materials	Approx yield (kg/ha)	Approx rate (lakh Rs/kg)	Amount (lakh Rs/ha)	Beneficiary	Value-added Product(s)	Approx yield (kg/ha)	Approx rate (Rs/kg)	Amount (lakh Rs/ha)	Beneficiary
1. Tomato	30,000	5.00	1.5	Farmer	Tomato paste	4500	40	1.80	Farmers/Industry/Consumers
					Lycopene concentrate / Functional food (salsa)	75/2000	1000/40	1.50	Industry/Consumers
2 Black carrot	25000	5.00	1.25	Farmer	Carotene concentrate	1000	175-	1.75	Farmers/Industry/Consumers
					Anthocyanin (natural colourants/ Functional food	5 -	10000 -	0.50 1.00	

3. Red Chilli	15000	6.00	0.9	Farmer	Capsanthin concentrate / Functional food	100 -	2000 -	2.5	Farmers/ Industry/ Consumers
4. Jamun	8000	10.00	0.8	Farmer	Fruit extract concentrate /Anthocyan in powder concentrate	150	1000	1.5	Industry/ Consumers
					Functional food/ seed concentrate / powder	-	-	0.60	Industry/ Consumers
5. Stevia	2000	50	1.0	Farmer	Quality leaf powder /Stevioside concentrate	100 -	2500 -	2.5 -	Farmers /Industry /consumers
6. Spirulina	100 kg (Spirulina biomass)	1000	1.0	Industry	Spirulina extract/ Spirulina concentrate/ pigment	20	12500	2.5	Industry/ Consumers

5. Research Achievements with Summary

Vegetables and fruits are rich source of health-benefiting natural antioxidants and food colorants such as vitamins, carotenoids, anthocyanins, flavonoids and related polyphenolics. Such constituents generally referred to as “nutraceuticals” are also known by different terminologies like, dietary supplements, functional foods, fusion foods, designer foods, value added foods etc. In addition to being nutritious, such ingredients aid specific bodily functions and provide health benefits of disease prevention through antioxidant activity and reduced disease risks. Besides vegetables and fruits, some microalgae and non-food crops are also recognized as a potential source of naturally occurring antioxidants and food colorants.

World-wide market for nutraceutical, food supplement and herbal medicinal products currently has surpassed US \$ 15 billion with the top markets being the United States of America (US \$ 3 billion), Europe (US\$ 2.4 billion), and Japan (US\$ 2.7 billion). According to an estimate, nutraceuticals market is expected to grow at the rate of 20%. Indian contribution in terms of domestic production and export to the global market is dismally low. At present Indian nutraceutical market is in its infant stage having its share in this market less than 1%. India, therefore, needs to be self sufficient in this sector

and must augment all resources at its command to increase the production of nutraceuticals and functional foods to meet both domestic and international demand. This can be made possible only if world-class quality extraction, isolation and purification procedures, and also the analytical methodologies are developed as per the international standards and specifications. Since perishable food commodities and nutraceuticals have a specific shelf-life, these run the risk of getting spoilt beyond a specific time. Food materials available in excessive quantities needs to be processed and converted into value added products to improve their shelf-life and nutrition value after fortification with nutraceuticals, natural antioxidants, food colorants, and other health supplements etc. The NAIP sub-project on “Value chain for the production of food grade nutraceuticals for use as natural antioxidants and food colorants” addresses the issue by ensuring availability of modern technical know-how for production of nutraceutical products derived from vegetables, fruits, microalgae and other non-food crops. The sub-project has identified nutraceutical-rich vegetables such as black carrot, tomato and chilli, and fruits like pomegranate and jamun as raw material for production of food grade nutraceuticals and functional foods. The project aims at developing technical know-how for processes/ products and protocols for efficient extraction, processing, purification, analysis (quality control), and formulation of food grade nutraceuticals and functional foods from vegetables, fruits, non-food crops. Under this NAIP sub-project following technologies have been developed and transferred to industry.

i) Technical know-how for the extraction and production of acylated anthocyanins concentrates from black carrot (*Daucus carota* L.)

Vegetable carrot is available in red, orange and /or purple/ black colour. The variety of pigments contained in them performs a range of protective functions in the human body. While orange carrots derive their color β -carotene, red carrots derive their color mainly from lycopene, a type of carotene believed to guard against heart disease and some cancers. Purple carrots possess an entirely different class of pigments—anthocyanins—which act as powerful antioxidants.



Such anthocyanins provide health benefits due to their significant antioxidant activity and their ability to promote immune system. The natural antioxidant and food colorant comprising of black carrot anthocyanins can be used to prepare nutraceutical enriched processed foods, RTS beverages (fruit drinks, alcoholic drinks), jams, candy, probiotics, and frozen foods like ice creams/yogurts, breads, cakes, candy, sauces and dietary supplements with pleasing pink/ purple colour and improvements in nutritional qualities.

Extraction, process standardization and analysis

Bench scale know-how has been developed for extraction of anthocyanins concentrate from black carrot juice or its pomace. Black carrots obtained from IARI fields were washed under cold tap water and cut into small pieces. The material was then extracted with acidified methanol. The macerate was filtered through cloth followed by Whatman No. 1 filter paper under vacuum. The residual biomass was re-extracted with the same solvent until most of the pigments were extracted. The combined extract was concentrated to obtain anthocyanin concentrate which is stored for further use under refrigerated conditions. The combined extract was passed through an ion exchange resins column and the column washed thoroughly with distilled water to remove sugar and other water soluble undesired materials. The pigments were eluted from the resin using acidified methanol/ethanol. The combined eluate was concentrated under vacuo in a flash evaporator at 35 °C almost to dryness. The concentrate has been further purified to obtain anthocyanins powder concentrate which can be used as a potential source of natural food colorant. The process of production of anthocyanin concentrate production is simple, cost effective and involves food grade solvents.

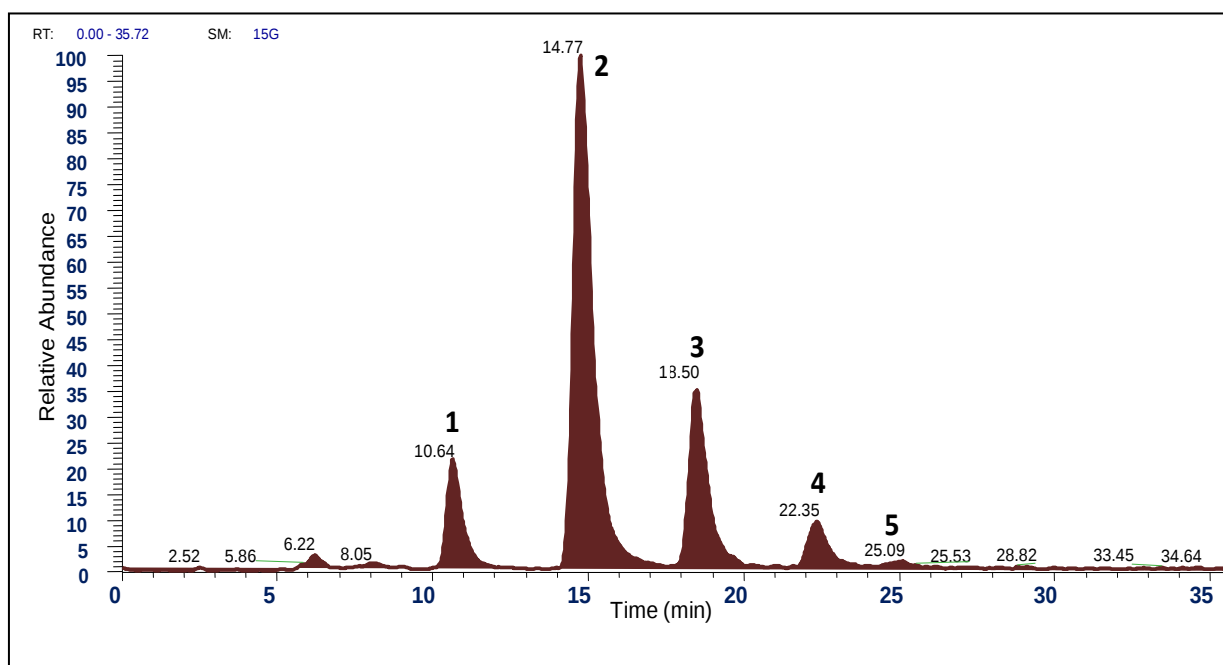


Fig. 1. TIC of Black carrot anthocyanin

Total five anthocyanins were identified by LC-ESI-MS in positive mode (Fig.1). The crystalline material was accurately weighed and dissolved in acetonitrile or the mobile phase to obtain 0.1% anthocyanins

solution. After appropriate dilution, the sample was subjected to LC/LC-MS analysis on RP-18 column. Three major peaks were cyanidin 3-xylosylglucosylgalactoside, cyanidin 3-xylosylgalactoside and cyanidin 3-sinapoylxylosylglucosylgalactoside were correspond to peaks 1, 2 and 3. While peaks 4 and 5 were present in trace amount which characterized as cyanidin 3-feruloylxylosylglucosylgalactoside and cyanidin 3-p-coumaroylxylosylglucosylgalactoside (Fig. 2).

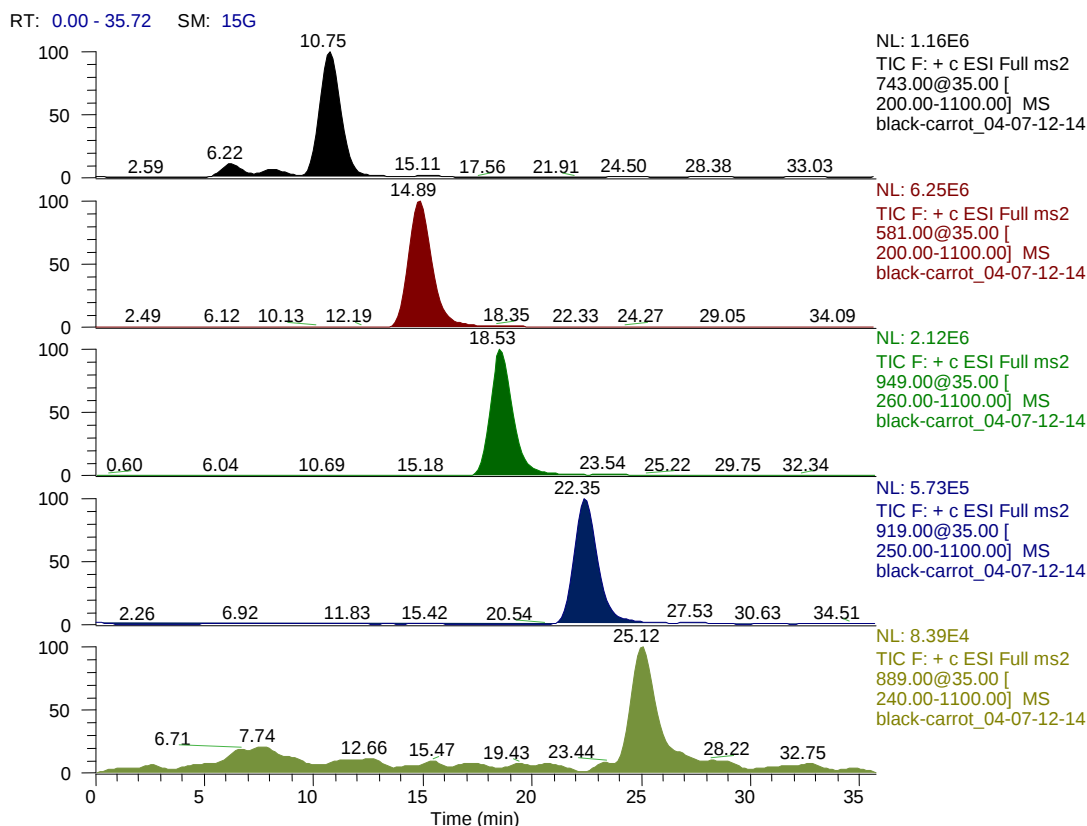


Fig. 2. Extracted ion chromatogram of anthocyanins from black carrot

Estimation of aroma compounds in black, orange and red carrot

Terpenoids, and especially the monoterpenes, have been shown to contribute significantly to the aroma and flavor of carrots and consequently carrot flavor is largely influenced by genetic variation. The monoterpenes are, in general, very volatile, and some loss of these compounds is therefore expected during processing of carrot. Variations in carrot types are also expected as the pigments are different in red, orange and black carrot. Variation in different mono and sesquiterpenoids are given below (Table 1). Characteristics aroma and flavor of particular carrot may be attributed to more concentration of a particular mono/sesquiterpenoids.

Table 1. Quantitative analysis of black, red and orange carrot root

Peak number	Retention time	Compound name	Black Carrot	Red Carrot	Orange Carrot
1	9.790	α -Pinene	0.13	0.03	-
2	10.543	Camphene	0.14	0.13	-
4	12.312	β -myrcene	-	-	1.09
5	12.476	β -Pinene	1.28	1.43	1.09
6	14.058	Cymene	0.28	0.05	0.43
7	14.257	Limonene	2.09	2.07	1.41
8	15.541	β -Ocimene	1.45	1.63	1.07
9	15.991	γ -Terpinene	0.93	1.3	0.99
10	17.707	α -Terpinolene	0.99	1.09	0.83
11	18.957	Nonanal	0.11	0.25	0.22
12	25.54	Decanal	-	0.15	0.11
13	27.389	Carvone	5.69	6.17	6.43
14	28.387	delta-3-Carene	12.60	17.84	18.33
15	42.500	Caryophyllene	1.48	1.02	6.06
16	44.923	β -Farnesene	0.46	0.17	0.16
17	47.323	Myristicin	4.46	--	--
18	47.673	Bisabolene	-	0.37	6.93
19	48.362	Elemicin	2.11	-	-

Similarly variations are also expected in the leaves of the corresponding carrots. Terpenoid profiles are almost similar in both leaves and roots.

Table 2. Quantitative analysis of black, red and orange carrot leaf

Peak number	Retention time	Compound name	Black Carrot	Red Carrot	Orange Carrot
1	6.377	α -Pinene	0.06	0.13	-
2	6.737	Camphene	0.32	0.010	0.06
3	7.281	β -Pinene	5.50	0.39	0.08
4	7.645	3-Hexen-1-ol, acetate	2.44	0.92	0.15
5	7.739	Cymene	0.81	0.04	0.09
6	7.956	Limonene	3.36	0.31	0.11
7	8.276	β -Ocimene	1.06	0.07	0.18
8	8.482	gamma.-Terpinene	0.32	0.03	0.01
9	9.301	Nonanal	0.54	0.08	0.02
10	11.870	delta 3-Carene	16.67	1.62	0.54
11	12.190	2-Decenal	0.25	0.02	-
12	14.439	β -Bourbonene	0.12	0.02	-
13	15.040	Caryophyllene	2.77	0.16	0.26

14	15.183	Zingiberene	0.09	-	0.02
15	15.524	Humulen	-	0.01	-
16	15.538	Farnesene	0.20	-	0.05
17	16.642	Myristicin	0.34	-	-
18	16.705	Cadinene	0.20	-	0.04
19	21.271	Farnesol	0.20	-	-
20	21.775	1-Octadecene	0.17	0.01	-
21	22.021	Nonadecane	0.22	0.03	-

ii) Extraction and production of anthocyanin concentrate from Jamun (*Syzygium cumini* L.)

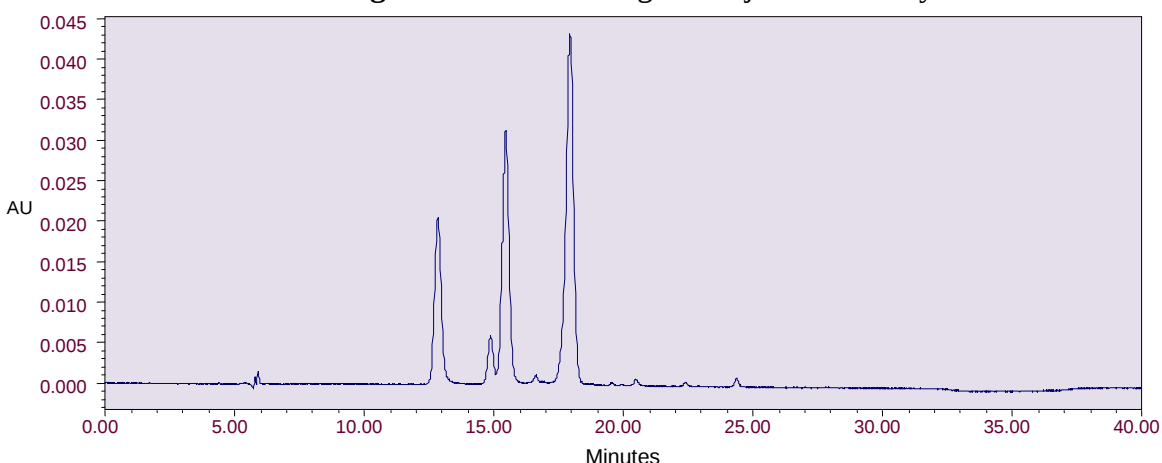


Jamun is a tropical and sub-tropical evergreen tree that yields purple ovoid fleshy fruit. It has remained an underutilized tropical fruit and is valued for its medicinal and therapeutic properties. In traditional Indian medicine, it is extensively used against diabetes and apart from that, it is also administered against various ailments like carminative, febrifuge, antibacterial, diuretic, and diarrhoea. Ethanolic extract of jamun kernel exhibited antioxidant activity superior to catechin which is attributed to the presence of anthocyanin content in the fruits. Fruits also yielded citric, malic and gallic acids. Seeds contain β -sitosterol. Diluted juice of fruits is diuretic and prevents enlargement of spleen. Seed powder of jamun is used as good diabetic medicine.

A method of making anthocyanin concentrates from jamun fruit skin, pulp or juice has been developed. The process can be briefly described as per the following details: (a) preparing extract of black carrot, ii) filtering and passing the extract through a column filled with a suitable adsorbent(s), iii) eluting the colorant from the absorbent with a suitable solvent, and iv) concentrating the eluate to provide anthocyanins concentrate. The concentrate has been further purified to obtain anthocyanin powder concentrate as a potential natural food colorant. The process of production of anthocyanin concentrate from jamun is simple, cost effective and involves food grade solvents.

As evident from HPLC analysis, jamun anthocyanin concentrate comprised of three major anthocyanin constituents (Fig. 3). On the basis of LC-MS-MS, these anthocyanins have been identified as i) myricetin 3-O-(4''-acetyl)- α -L-rhamnopyranosides, ii) delphinidin-3-gentiobiside and iii) malvidine-3-laminariboside. Other two minor anthocyanins include: myricetin 3-O-(4''-acetyl) - α -rhamnopyranosides and delphinidin-3-gentiobiside. These anthocyanins in mixture form exhibit excellent antioxidant activity.

Fig 3. HPLC chromatogram of jamun anthocyanins



iii) Know-how for the production of capsaicinoids and capsanthin concentrates from Red Chilli (*Capsicum annum* L.)

Chillies are the dried red fruits of the genus *Capsicum* and family Solanaceae. The two well known species are *Capsicum annum* L. and *C. frutescens* L., the former being widely grown and economically important of the domesticated chilli peppers. India is the largest producer and exporter of chillies. Pungency and color are the two main quality attributes in chillies. The red color of chillies is due to the presence of the carotenoid pigment like capsanthin (major, 35%), zeaxanthin, violaxanthin, cryptoxanthin, β -carotene etc. These pigments are present in chillies mainly in the esterified form and to a small extent in non-esterified forms. The hot flavour of chillies is due to the presence of two major closely related capsaicinoids namely capsaicin and dihydrocapsaicin that account for approximately 90% of the pungency. Burning and painful sensations associated with capsaicin results from its chemical interaction with sensory neurons and depletion of neurotransmitters. While capsanthin concentrate is used as natural colorant in beverages, pickles, snack foods, salad dressing, seasoning and confectionery industry etc., capsaicinoid concentrate find application as flavouring agent that cause burning (hot) sensation.



It also find application in pharma industry as anti-inflammatory agent and used in tropical ointments in concentrations to relieve minor aches, muscle pains, joint pains, back ache, peripheral neuropathic pains, osteoarthritis pain as well as strains and sprains.

Extraction, process standardization and analysis

In the spice extraction industry, chilli oleoresin is produced by extraction of chilli powder with GRAS solvents followed by desolventization to get deep colored oleoresin. Selective enzyme mediated extraction using alcohol as the solvent has also been employed as a viable technique. An improved process has been developed for production of standardized chilli oleoresins and concentrates containing food colorant – capsanthin, and pungent principles capsaicinoids. The process is simple, cost effective and involves use of food grade solvents for extraction.

The process starts with cleaning to remove any extraneous matter in chilli powder from red chilli. This is followed by drying to bring down the moisture to nearly 5%. The crisp dry chilli was subjected to grinding or passed through a hammer mill with a suitable sieve in order to get a mixture of pericarp flakes and seeds.

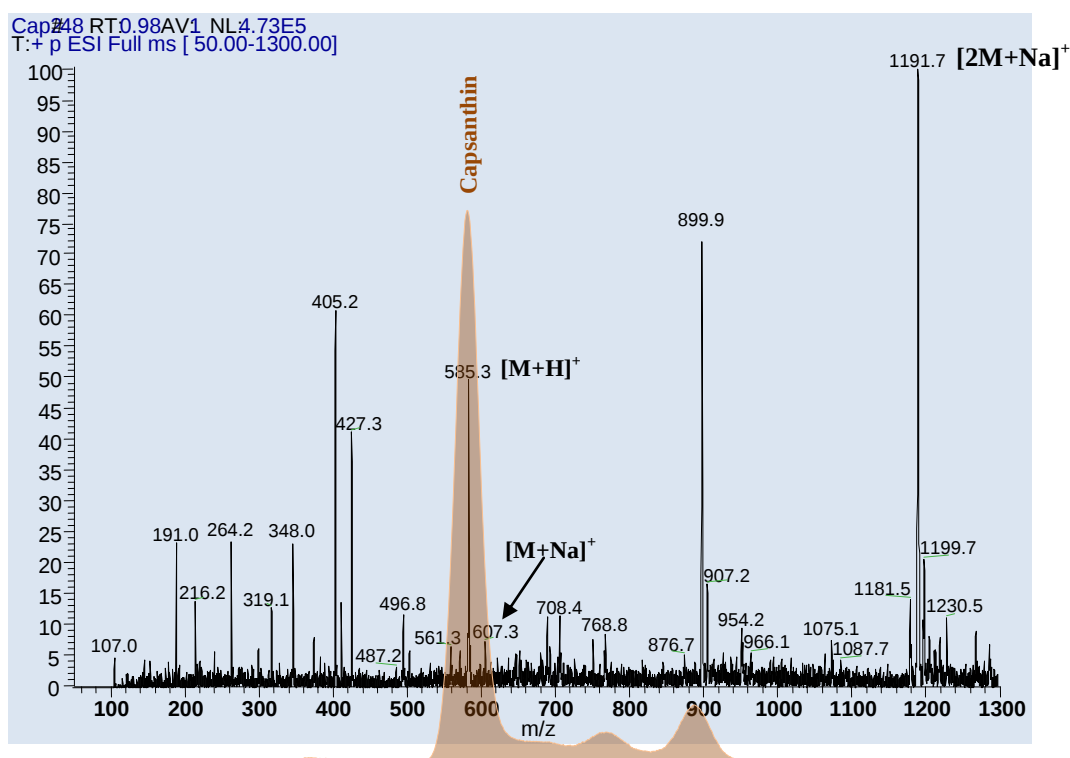


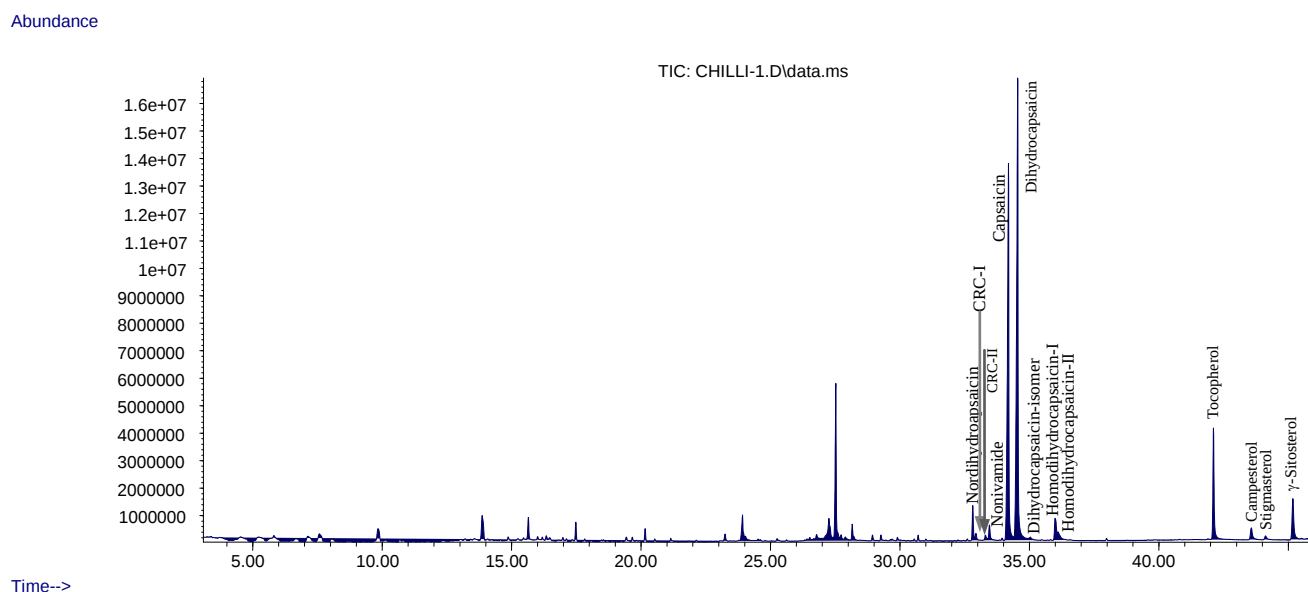
Fig. 4. HPLC chromatogram and mass spectrum of purified capsanthin

This mixture was passed through a smaller mesh sieve to separate flakes of pericarp free of seeds. The powdered chilli/paprika flakes was soaked in hexane and stirred for half an hour or subjected to sonication for 10 minutes. The stirred material was then loaded in a glass column and extracted with hexane, acetone or a solvent mixture containing acetone hexane. The resultant extract(s) was desolventized under vacuo at temperature not exceeding 50 °C to remove solvent. Hexane extract was saponified with methanolic KOH

(5-10%) to obtain capsanthin. The material remained after extraction with hexane was subjected to further extraction with hexane + acetone or acetone to obtain capsaicinoid rich extract concentrate which was analyzed by HPLC. The method uses comparatively less solvent, reduced processing time and higher yield of the resin. Pure capsanthin was separated using flash chromatography by sequential increase in polarity of solvent. Separated capsanthin was characterized by LC/MS (Fig. 4).

The purified chilli/paprika oleoresin was accurately weighed to obtain 0.1% chilli oleoresin solution. A simple and rapid method was developed including extraction of capsaicinoids as well as sterols and tocopherol with acetonitrile, followed by GC/MS analysis (Fig. 5). This procedure allows simultaneous separation of capsaicinoids, tocopherol and sterols. The mean recovery was between 90.6–99.7%.

Fig 5. TIC of identified capsaicinoids and other phytochemicals in chilli



Besides major capsaicinoids (capsaicin, dihydrocapsaicin, nordihydrocapsaicin), four more minor capsaicinoids (nonivamide, nornordihydrocapsaicin, homodihydrocapsaicin I and II) were also detected in chilli samples. α -tocopherol was also detected in same run along with three phytosterols (campesterol, γ -sitosterol and stigmasterol). The method is suitable for capsaicinoids, vitamin E and phytosterols determination from any type of red peppers and effective for rapid screening for its nutraceutical composition using single solvent extraction. The present method can be explored for large scale screening of chili cultivars for the development of nutraceutical rich chili cultivars. The method was validated using four different chilli cultivars and the variation in the content of different capsaicinoids were observed (Table 3).

Table 3. Quantity and relative content* of capsaicinoids in fresh chili varieties analysed by GC-MS

	Rt (min)	m/z	Chilli- I	Chilli -II	Chilli -III	Chilli- IV
NDHC	32.814	293	-	1.05	0.42	2.94
CRC-I	32.935	293	q	nd	nd	nd
CRC-II	33.310	299	q	q	nd	nd
NVM	33.437	293	0.32	1.51	-	1.55
NNDHC	33.950	279	d	nd	nd	nd
C	34.193	305	1.29	0.55	-	0.65
DHC	34.550	307	-	0.33	0.23	0.18
DHC-i	35.042	307	nq	q	nq	nq
HDHC-I	35.994	321	2.18	4.38	-	0.70
HDHC-II	36.114	321	nq	nq	nq	nq
Tocopherol	42.113	430	19.10	-	8.93	4.39
Campesterol	43.576	400	2.54	0.57	-	0.70
Stigmasterol	44.125	412	q	nq	nq	nq
γ -Sitosterol	45.853	414	-	4.05	2.40	2.69

q:quantifiable; nq:non quantifiable; nd: non detectable

*relative content = (area of sample-area of sample with least value)/ area of sample with least value

Method described in this study is simple, rapid, and allows high recovery. Thus the estimation method of capsaicinoid, tocopherol and phytosterols capsaicinoids made this technique unique and ideal for quick screening of chilli lines used for breeding programme or nutraceutical exploitation (Table 3 & 4). The method ensured high reproducibility. The use of GC/MS for quantitation gives information about the possible interferences and allows a more accurate quantitation of the capsaicinoid, tocopherol and sterol content. The method is suitable for capsaicinoids determination from any type of peppers and effective for rapid screening of peppers for its nutraceutical composition using single solvent extraction.

Table 4. Dihydrocapsaicin and capsaicin profile in different strains/varieties collected

Strains /Varieties	Fruit Stages	Dihydrocapsaicin content (dwb) %	Capsaicin (dwb) %
North Eastern	Green	0.3844	0.3111
	Half ripe	0.4755	0.4245
	Ripened on plants	0.4851	0.5559
	Mature red	0.4084	0.6423

Local collection	Green	0.2931	0.3511
	Half ripe	0.2220	0.3182
	Ripened on plants	0.0940	0.1603
	Mature red	0.0855	0.1415
UHF	Green	0.5129	0.2861
	Half ripe	0.4648	0.2915
	Ripened on plants	0.2567	0.1818
	Mature red	0.2295	0.1436
Kashmiri	Green	0.1344	0.1055
	Half ripe	0.1513	0.1343
	Ripened on plants	0.1614	0.1245
	Mature red	0.1103	0.0973
Paprika	Green	Absent	Absent
	Half ripe	Absent	Absent
	Ripened on plants	Absent	Absent
	Mature red	Absent	Absent

iv) Technical know-how for the extraction and production of lycopene concentrate from tomato

Lycopene is the vibrant red pigment and a fat soluble carotenoid. It's a major carotenoid in tomato (*Lycopersicon esculentum* Mill.) fruit. It serves as an intermediate for the biosynthesis of other carotenoids and is found in moderate to high concentrations in such foods as tomato, watermelon, red grapefruit, capsicum, apricot and red guava. As compared to its biosynthetic derivatives such as β -carotene, lycopene is two times more efficacious free radical scavenger, and its presence in the diet positively correlates with reduced cancer incidence. Since lycopene has value as a phytonutrient, many breeders want to maximize lycopene content in their breeding lines, and growers want to utilize production methods to increase lycopene content. Upon growing interest on nutraceutical research, crop improvement for increased fruit lycopene content requires a rapid and accurate method of lycopene quantification. Among the various available techniques, high-performance liquid chromatography (HPLC) can be accurate, however, it is laborious, requires skilled labor and costly solvents. Similarly, spectrophotometric methods, although easier than HPLC, also require time-consuming extractions and may not be as accurate as HPLC, as they often overestimate fruit lycopene content.

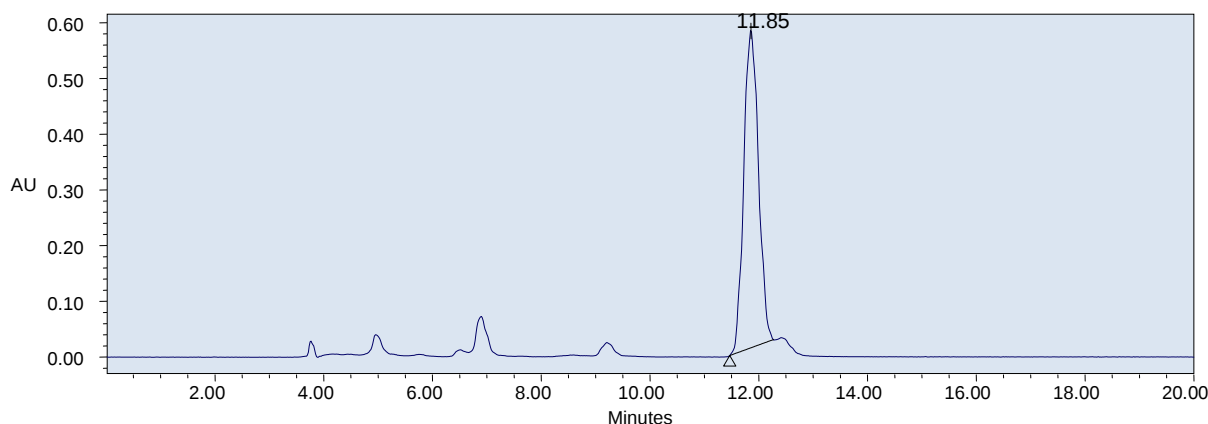
Extraction, process standardization and analysis

Total procedure was done under diffused light and in amber colour glass apparatus. Tomato (5 g) was extracted with 50 mL of hexane: acetone (1:1) in a beaker using homogenizer/ultrasonicator and the

solvent was pooled. Extraction was done three times or more until the tomato material become colourless. Solvent was transferred into a separatory funnel and 10 mL water was added. The solvent mixture will separate into two layers with lycopene will be in upper hexane layer. Extraction was done 2-3 times and lower layer was discarded. Upper hexane layer was collected and evaporated using a rotary evaporator. After evaporation, lycopene was dissolved in tertiary butyl methyl ether (TBME) and injected in HPLC for quantification.

20 μ l of extract is injected in HPLC and run for 30 minutes. Carotenoids were analysed using a Waters HPLC system (Milford, MA, USA) connected to an PDA detector with $\lambda_{\max}$ set at 453 nm using a gradient method (Fig. 6). HPLC conditions: Stationary phase: C30 carotenoid column (YMCe, 150 X 4.6 mm, 5mm; Waters, Milford, MA, USA); Mobile phase: MTBE:Methanol (70:30), isocratic; Flow rate: 1 ml min⁻¹; $\lambda_{\max}$: 471 nm; Detector: PDA; Retention time: 12.0 min

Fig 6. Chromatogram of lycopene rich extract of tomato



v) **Technical know-how for the extraction and production of phycocyanin concentrate from *Spirulina***

The cyanobacterium *Spirulina* is a blue-green microalga with a long history of food source in various countries. It has been used by humans because of its nutritional and possibly medicinal effects. Since it is very rich in protein (60-70%) and other nutrients, it is currently used as a health supplement and as a treatment for malnutrition. Besides being a rich source of proteins, *Spirulina* contains 10-20% of the blue pigment phycocyanin by weight and this makes it the best natural blue color for foods. The *Spirulina* pigment phycocyanin has antioxidant, anti-inflammatory and hepatoprotective properties. β -Carotene, a natural pigment which acts as an antioxidant for vitamin A, has also been reported in higher amounts in *Spirulina* compared to other vegetable sources. A blue food color from *Spirulina* called “Lina blue” containing 17% phycocyanin is used as colorant in chewing gum, ice sherbets, frozen confectioneries, candies, soft drinks and dairy products. Commercial tablets of *Spirulina* in the name of Spiruwin and Spirulina are available from Baidyanath and Perry Nutraceuticals. Some Japanese companies use these natural pigments in eyeliners, eye shadows and lipstick foundations.



Influence of incubation time on different growth parameters, enzymes assay and pigment profiles have been examined. The dry weight of the biomass varied from the lowest of 3.55 to the maximum of 8.54 mg ml⁻¹. Chlorophyll and carotenoid content varied between 2.46-24.65 µg ml⁻¹ and 0.961-1.534 µg ml⁻¹ respectively. Phycobilins (phycocyanin, allophycocyanin and phycoerythrin) which are unique in cyanobacteria also varied distinctly amongst different strains of *Spirulina*. Maximum total soluble proteins varied between 36.59 -108.02 µg ml⁻¹.

Table 5. Purity and recovery of phycocyanin at different steps of purification

Step	Volume	PC(µg/ml)	Purity(OD ₆₂₀ /OD ₂₈₀)	Recovery (%)
Crude extract	200	77.4	0.75	100
Ammonium Sulphate pptn	10	123.8	1.5	80
Dialysis	10	601.4	2.93	39
DEAE Column	5	413	4.58	14

The total carbohydrates showed variation with incubation time. The efficient strains of *Spirulina* (CCC 540, P&A, CCC 480 ,CCC 482) in terms of growth potential and pigment profile have also been identified.

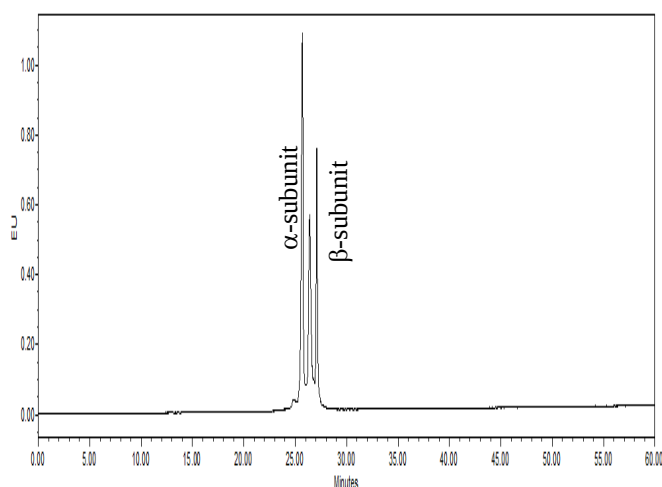


Fig 7. HPLC chromatogram of phycocyanin

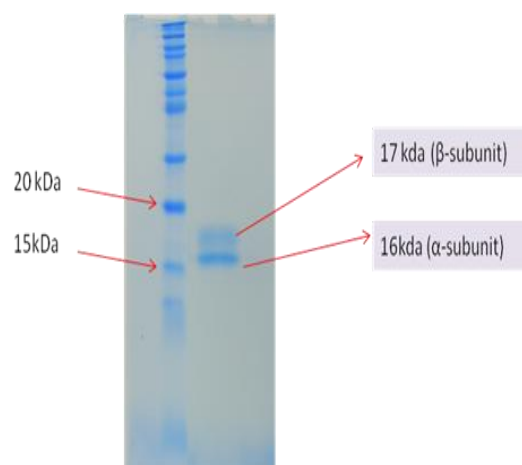


Fig 8. SDS –PAGE analysis of phycocyanin

Protocol for enhanced production of pigments (mg/g dry weight) from *Spirulina* was standardized by varying environmental variables (light intensity, temperature, CO₂ level, pH and NaCl concentration) for mass production during exponential phase. Phycocyanin content enhanced significantly at 35°C and there was a variable response towards change in carbon dioxide concentration and pH change. Chlorophyll and carotenoids exhibited variable pattern with increase in NaCl and its higher concentration enhanced phycobilins. The spirulina extract was purified as per the literature method (Table 5). Purified extract was analysed using C₅ column and PDA and fluorescence detectors for the HPLC analysis. The results indicated the presence of α and β subunits for phycocyanins isomers.

Table 6. β -carotene content in different strains of spirulina

Strain No.	β -carotene (mg 100g dry weight ⁻¹)	Strain No.	β -carotene (mg 100g dry weight ⁻¹)
477	183.71	483	215.38
478	229.51	538	220.16
479	239.01	539	225.26
480	225.02	540	260.39
481	216.61	P&A	289.10
482	237.98	-	-

Ratio at two wavelengths viz 640nm and 280nm indicate the identity of two subunits (Ratio is 1 for α and 2 for β subunit). SDS PAGE analysis confirmed the presence of α and β subunit for purified phycocyanins. PCR amplification confirmed the presence of *cpcB-IGS-cpcA* gene responsible for phycocyanin synthesis (Fig 7&8). *Spirulina* strains were analysed for β -carotene estimation (HPLC) and it was found that the content varied between 183.7 to 289.1 mg 100g dry weight⁻¹ (Table 6).

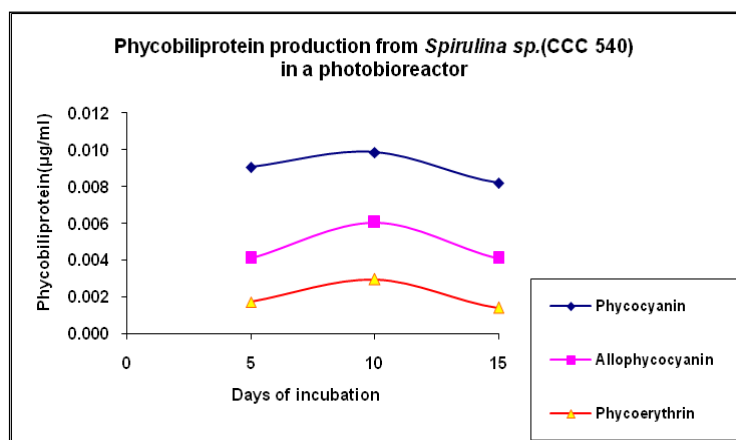


Fig. 9. Variation of phycobilin production upon incubation

The growth of *Spirulina* sp. (CCC 540) was compared in photobioreactor system *vis a vis* stirred raceways lodged in a polyhouse. Cell density reached a higher value in a photobioreactor under controlled conditions as compared to its cultivation in high volume raceways. There was a gradual increase in chlorophyll and carotenoids upto 15 days of incubation, and phycobilins were maximum after 10th day of incubation followed by a decline thereafter (Fig. 9).

Extraction, process standardization and analysis

Spirulina biomass (commercial grade) procured from the industry (Hydrolina limited Chennai) was extracted with water to isolate *phycocyanin*. *Spirulina* biomass was suspended in 200 ml of acidified water (RO or distilled) and the mixture was kept in a shaker incubator for one to three hour at the specified temperature. After extraction the suspended material was filtered to obtain the extract and the remaining biomass again extracted as above. The filtrate was centrifuged for 10 minutes to remove suspended particles in the form of a pallet and the supernatant was collected in an amber colored flask. The combined supernatants were combined and concentrated under vacuo at temperature not exceeding 40 °C to obtain phycocyanin liquid concentrate (Product 1). The resultant concentrate was subjected to freeze drying in a lyophilizer to obtain phycocyanin concentrate in the powdered form (Product 2). Phycocyanin content in *Spirulina* extract powder concentrate was measured by spectrophotometry and/or reversed phase HPLC. *Spirulina* residual biomass so obtained was dried to obtain *Spirulina* powder which is devoid of unpleasant odour. It can be used as raw material for making *Spirulina* protein supplement tablets/capsule.

vi) Technical know-how for the extraction and production of rebaudioside rich natural sweetener from *Stevia rebaudiana*



Stevia rebaudiana is the naturally occurring only plant having both sweetening and anti-diabetic property. It is one of several members of the genus *Stevia* and one of only two that produce sweet steviol glycosides. It is suitable to diabetic patients as well as for obese persons intending to lose weight. Originally cultivated in South America (Brazil and Paraguay) it and is currently domesticated in China, Japan, India and several other countries. Leaves of *Stevia rebaudiana* Bertonii have been used to sweeten tea for centuries and for the last 20 years in countries of South America and Southeast Asia. The sweetness has been attributed to the glycosides stevioside, steviolbioside, and rebaudiosides A, B, C, D, E,

F, and dulcoside A. Stevioside is 300 times sweeter than sugar but has a bitter after-taste. Stevioside was between 110 and 270 times sweeter than sucrose, rebaudioside A between 150 and 320, and rebaudioside C between 40 and 60. Dulcoside A was 30 times sweeter than sucrose.

Table 7. Variation in stevioside and rebaudioside-A contents in different plant parts of *S. rebaudiana* with plant growth and development

Month of harvesting	Plant growth Stages	Stevioside content (%)			Rebaudioside-A content (%)		
		Leaves	Green stem	Woody stem	Leaves	Green stem	Woody stem
March	Vegetative	6.16(2.48)	0.68(0.83)	na	3.58(1.89)	0.53(0.72)	na
April	Vegetative	6.21(2.49)	0.42(0.65)	na	3.81(1.94)	0.29(0.54)	na
May	Vegetative	7.08(2.66)	0.31(0.56)	na	4.86(2.20)	0.19(0.44)	na
June	Vegetative	8.55(2.92)	0.41(0.64)	0.17(0.42)	6.41(2.53)	0.47(0.69)	0.12(0.35)
July	Vegetative	8.16(2.86)	0.50(0.70)	0.20(0.45)	6.37(2.52)	0.71(0.84)	0.13(0.36)
August	Vegetative	8.20(2.86)	0.72(0.85)	0.22(0.47)	7.00(2.65)	0.85(0.92)	0.15(0.38)
September	Flowering	6.72(2.59)	0.82(0.91)	0.33(0.57)	3.39(1.84)	0.93(0.97)	0.18(0.43)

Rebaudioside A was the least astringent, the least bitter, had the least persistent aftertaste and was judged to have the most favourable sensory attributes of the four major steviol glycosides (Tanaka 1982, 1997). Relative to other high potency sweeteners such as aspartame, bitterness tends to increase with concentration for both stevioside and rebaudioside A. Both stevioside and rebaudioside A are synergistic in mixtures with other high potency sweeteners such as aspartame and are good candidates for inclusion in blends (Schiffman et al. 1995). Although specialty applications may exist for the other glycosides, increasing levels of rebaudioside A in stevia leaves is a clear objective for breeding work. Most of the commercial processing of stevia leaves occurs in Japan and there are dozens of patents describing methods for the extraction of steviol glycosides. Stevia sweeteners have a long history of use in South America and Japan without any adverse effects. Nonetheless, the safety of stevia sweeteners has been the subject of controversy for some years.

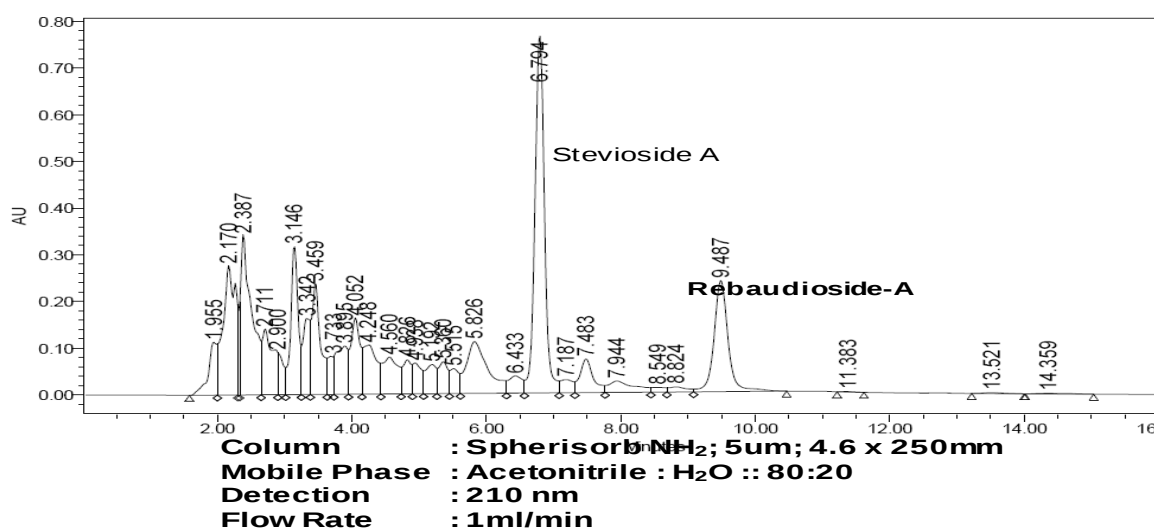
Stevia represents a new opportunity for researchers and farmers alike. The worldwide demand for high potency sweeteners is expected to increase especially with the new practice of blending different. In addition, the sweet steviol glycosides have functional and sensory properties superior to those of many other high potency sweeteners. Stevia is likely to become a major source of high potency sweetener for the growing natural food market in the future. Variation in stevioside and rebaudioside-A contents in different plant parts of *S. rebaudiana* was recorded with plant growth and development (Table 7).

Extraction, process standardization and analysis

Process has been refined for production of steviol glycoside powder concentrate from *Stevia rebaudiana* leaves. The process is simple, cost effective and involves following steps:

The dried Stevia leaves powder was suspended in solvent comprising of one or more of solvents comprising of water, acetone methanol, ethyl alcohol and /or isopropyl alcohol. The suspension was stirred at temperature ranging from 40-70 °C. The stirring was continued for 2-6 hrs after which the suspension was filtered. The biomass left after filtration was again suspended in solvent(s) and extraction repeated one more time as above. The filtrate was combined and its pH was adjusted to desired pH by adding dilute HCl, NaOH or calcium hydroxide solution. The mixture was then stirred and made neutral. It was then filtered and the filtrate concentrated under vacuo. The concentrate was then dissolved in solvent and the mixture decolorized by passing through suitable adsorbents (acidic, basic, neutral). The eluate was then concentrated under vacuo and then crystallized to obtain Stevia powder. Alternatively after removal of solvents, the sweet compounds were separated through column chromatography (authenticated through TLC comparison with reference compounds). The product was further re-crystallized to obtain white Stevia powder and the product analyzed for its purity by HPLC (Fig 10). Besides extraction and separation of pure steviol glycosides, attempts have also been made to separate the bitter principle, stevioside without using column chromatography.

Fig 10. HPLC analysis of *Stevia rebaudiana* extract concentrate



vii) Novel micro and nano-sized nutraceutical formulations with increased efficacy

a) β -carotene incorporated polymeric materials and their water soluble micro/ nano particles

β -carotene, a phytochemical pigment is known to exhibit powerful antioxidant and other effects. It mainly occurs in orange/red carrots. It is thought to be a form of vitamin A that is quickly converted into vitamin A inside the body. β -carotene supplements provide health benefits to people with specific health problems of vitamin A deficiency, macular degeneration, osteoarthritis etc. Efforts have been

made to develop amphiphilic polymer based nano-formulation of β -carotene for slow release, higher bioavailability and higher stability than the existing formulations. Due to its nano size the encapsulated product is more effective than β -carotene as it not only extends the release time but also increase absorption in human body. Such formulations score better than others as they do not require a surfactant or a carrier. The polymer contained therein acts both as surfactant and a carrier. The synthesis of such polymers and preparation of encapsulated product is cost effective cheap raw materials and excludes the use of solvents thus make the synthetic procedure environmentally benign. Such nano-formulation provide required amount of β -carotene with greater bioavailability in the human body. Incidentally this is the first report of synthesis and development of amphiphilic polymer based nano-formulations and their use in encapsulation of β -carotene.

b) Phytosomal complexation of black carrot and jamun anthocyanins

Black carrot and jamun anthocyanins are readily water soluble and limited lipid solubility. After ingestion by the human/animal systems, a significant part of the anthocyanin is excreted out. Thus the health benefitting effects of the anthocyanin enriched nutraceutical product is not realized fully. It was therefore considered desirable to check/regulate solubility of black carrot and jamun anthocyanins. Since naturally occurring phospholipids are both water and lipid soluble because of hydrophilic head and hydrophobic tail, attempts were made to conjugate polar black carrot/jamun anthocyanins with hydrophilic head of phospholipids to prepare phytosomal complexes. Complexation has been confirmed by ^{31}P NMR spectroscopy. Such complex has restricted water solubility and enhanced lipid solubility. Thus the antioxidant anthocyanin is slowly released from the phytosome complex. The restricted solubility and slow release of anthocyanin make it more bioavailable. Such nano-sized micelles of phytosomal complexes exhibit enhanced health benefitting effects.

Cyclodextrin inclusion complexes of black carrot and jamun anthocyanin have also been developed. The particle size of cyclodextrin inclusion complex with black carrot anthocyanin (716 nm) and jamun anthocyanins (692 nm) is comparable to the particle size of pure black carrot anthocyanin crystal (503 nm) and jamun anthocyanin crystal (353 nm). Scanning electron microscopy (SEM) studies revealed that black carrot anthocyanin has irregular crystal particles whereas β -cyclodextrin crystal (polyhedral) has cavity structure. Black carrot anthocyanins- β -cyclodextrin inclusion complex (IC) on the other hand appeared as a compact and homogeneous plate like structure. Absence of cavity in the IC confirmed its structure. Unlike Jamun anthocyanin which under SEM appears as irregular shaped crystal particles, maltodextrin has a spherical structure (Fig. 11). Maltodextrin encapsulated jamun anthocyanins on the other hand results in a homogeneous sheet like structure. Absence of spherical maltodextrin and irregular appearance of sheet like structure of jamun anthocyanin confirmed the encapsulation of anthocyanins with carrier maltodextrin. Such products being more effective can be directly used to develop black carrot and jamun anthocyanin enriched functional foods.

c) PEG and dextrin based water soluble capsanthin concentrates

Red chilli (paprika) derived capsanthin is a widely used natural pigment and food colorant. Being lipophilic it is lipid soluble limited solubility in water. For a natural food colorant it must be water

soluble. Encapsulation of capsanthin oleoresin has been achieved with dextrin and PEG based polymers employed in different ratio. A recipe of water soluble capsanthin has been standardized with a suitable polymeric formulant. The formulated capsanthin is considerably water-soluble and can find wide application in food industry.

d) Encapsulated phycocyanins with decreased water solubility

Like anthocyanins, Spirulina derived blue pigment – phycocyanin is highly soluble in water and consequently significant portion of phycocyanin after ingestion is excreted out. Encapsulation with polymer matrices is therefore desired to limit its water solubility. Attempts have been made to develop encapsulated products of phycocyanin by complexation with maltodextrin, guar gum, gum arabic, and /or chitosan by freeze drying. The ratio of polymer to phycocyanin concentrate has been optimized to obtain free flowable powder.

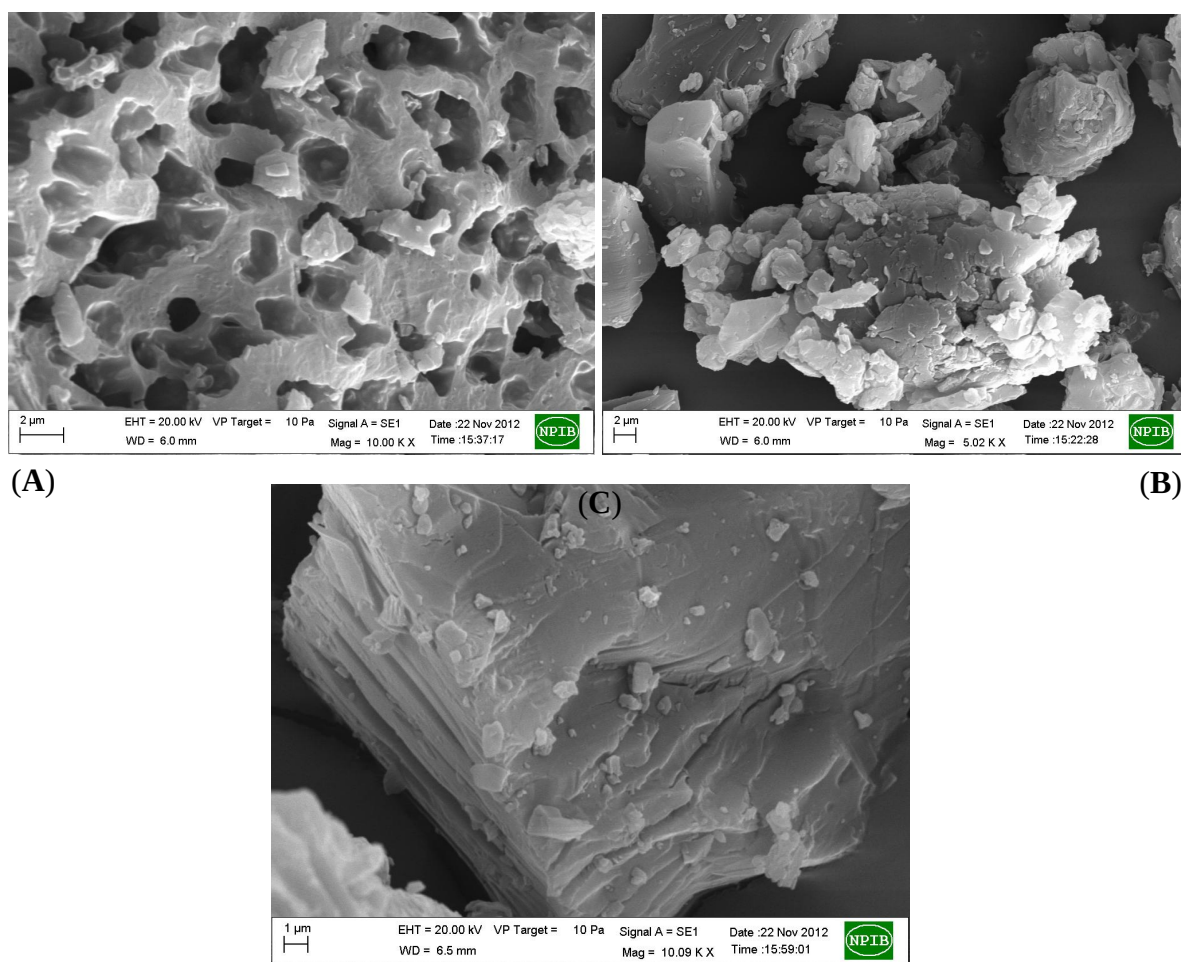


Fig 11. SEM picture of **A:** b-cyclodextrin **B:** black carrot anthocyanin **C:** inclusion complex

The solubility of phycocyanin in water has been restricted and considerably reduced by encapsulation to optimize its bioavailability to the biological system. The encapsulated phycocyanin so developed is more active and have thus wider application in preparation of nutraceuticals, functional foods and pharmaceuticals

viii) Nutraceutical processing, enrichment and functionalization

Growing consumer demand in replacing synthetic dyes in processed products has triggered interest in anthocyanin rich ingredients as natural colorants. Health-conscious consumers are increasingly seeking functional and fusion foods in an effort to control their health and well being. Considering the immense potential of nutraceuticals and functional foods in improving wellness and health, efforts have been made to incorporate the targeted nutraceuticals in the fusion products. Accordingly know-how for phytochemical supplements and functional beverages with anthocyanins, flavonoids, and lycopene from carrots, tomatoes, chilli, Spirulina etc. has been developed. Standardization of functional beverages utilizing targeted bioactive molecules such as lycopene and anthocyanins from vegetables and fruits for blending into fruit juice system has not only led to improvement in both enhancements in stability and product quality. The process for the production of nutraceutical-enriched guava, grape and mango pana RTS and aonla candy functionalized with black carrot anthocyanins as well as capsicum-tomato salsa (capsitom) and bakery products is briefly described below:

- a) **Red Guava Nectar:** Guava based Ready –To- Serve drinks are nutritious and high in antioxidants. Guava beverages available in the market are usually available in white and red variants. Depending upon the natural colorant used, the anthocyanin content in the product ranges from 50-100mg/L and has high stability. The anthocyanin pigment apart from conferring are usually colored with synthetic color (E-224) which can be harmful to human body. Because of growing safety concerns, consumer is increasingly looking for natural GRAS (generally recognized as safe) status products. The newly developed product viz. Red guava nectar contains natural acylated anthocyanins which doesnot require E-labelling. characteristics pink color to the product also imparts health promoting capacity to the drink
- b) **Anthocyanin enriched grape juice:** Unlike grape juice made from black/purple grapes which are nutraceutical rich, the juice extracted from green grapes lack sensory appeal. Efforts were therefore made to enrich green grape juice with black carrot anthocyanins.
- c) **Nutri-mango pana:** Mango based Ready –To- Serve drinks are nutritious and high in antioxidants. Mango pana available in the market are usually available in natural variants. Because of growing health concerns, consumer is increasingly looking for natural GRAS (generally recognized as safe) status products. The newly developed product viz. nutr-mango pana contains natural acylated anthocyanins which does not require E-labelling. Depending upon the natural colorant used the anthocyanin content in the product ranges from 50-100mg/L and has high stability. The anthocyanin pigment apart from conferring characteristics pink/purple color to the product also imparts health promoting capacity to the drink
- d) **Red amla candy:** Novel red amla candy combines the biologically active components from amla and black carrots. Amla fruits are known for pharmaceutical applications in ayurvedic system and are high in ascorbic acid, ellagitannins and other phenolics. Black carrots are an exceptionally good source of acylated anthocyanins and possess high antioxidant activity. Red amla pieces in syrup enriched with natural colorant has high stability for anthocyanins for 9 months at low temperature. Dried candy processed through DIS process, dried and vacuum packed have good

shelf for 6 months. Incorporation of natural red anthocyanin provides a high sensory appeal to the amla products.



Red guava nectar



Novel red amla candy



Black carrot squash

e) Nutraceutical-enriched capsicum-tomato salsa/puree

Large scale commercial production of red capsicum and tomato under north Indian conditions has been made possible due to adoption of green house technology (cultivation of vegetables under controlled conditions) by farmers. The Red capsicum sells at a premium price ranging from Rs 100-150 per kg during the early season and is primarily eaten as fresh salad or for cooking purposes. Tomato is also produced in large quantities throughout the country and during the peak season there is a huge glut because of over production and farmers do not fetch the premium price which they usually get in the early season. In order to get premium price for these crops, efforts have been made to process these vegetables to get value added product which can be made available through out the year.

Freshly harvested red capsicum and tomato were processed for developing a value-added salsa, thick sauce like product. Fresh fruits were subjected to hot break at 90° C for 20 minute (HTST process) and passed through pulper. The homogenized pulp was processed and enriched with flavors and other minor ingredients such as sugar, salt, pepper, garlic, onion powder etc. The puree was cooked to a desired consistency till it reached a ° brix level of 25°C and final product preserved with class II preservatives.



Capsicum-tomato fusion product

f) Nutraceutical-enriched baked products

Black carrots known for their high anthocyanin pigments and have immense potential as a source of natural food colorant. Yet they are underutilized and their commercial importance remains unknown. β -carotene is another rich source of antioxidant. Carrots are the one of the best source of carotenoides.

The technology developed employs heat treatment followed by cell wall degrading enzyme for recovering higher amounts of phenolics, and anthocyanins. Extraction method has been developed for carotenes from red/orange carrot for fortification.



Anthocyanin and carotene enriched bread



Anthocyanin enriched biscuit

ix) Transfer of technology to industry

Success of the project can be further vouched from the fact that technologies developed under the project have been transferred to two industries for commercialization. The host institute (IARI New Delhi) has entered into an MOU with the companies namely i) Ozone Biotech Faridabad, and ii) M/s Pratistha Industries Secunderabad at a total consideration of 15.5 lakh (7.5 + 8.0 lakh, non-exclusive). These industries are in the process of setting up the plant for commercial production of high-value nutraceuticals and functional foods.



6. Innovations

Under this NAIP sub-project several new innovative products, processes and technologies have been developed for the production of nutraceuticals and functional foods from food and non-food crops. These include:

1. Natural antioxidants and food colorants such as i) anthocyanins from black carrot and jamun, capsanthin concentrate from chilli (paprika), ii) lycopene concentrate from tomato, iii) phycocyanin concentrate from Spirulina.
2. Know-how has also been developed for the production of rebaudioside-A enriched Steviol glycodide from Stevia which has less bitter after-taste and hence more acceptable.
3. Novel extraction techniques namely ultra-sonication, accelerated solvent extraction, super-critical fluid extraction and enzyme mediated extraction have been employed for enhanced extraction and recovery of nutraceuticals.
4. Analytical protocols (HPLC, GC-MS, LC-MS-MS) have been standardized for the estimation, analysis and quality control of active ingredients in technical materials and finished products.
5. Efforts have been made to regulate solubility and hence increase bioavailability of lipophilic and hydrophilic nutraceuticals through encapsulation and/or complexation. Key technologies have been made available to encapsulate anthocyanins and phycocyanin with dextrin, maltodextrin, guar gum, chitosan etc. and /or complexation with natural phytosomes (phospholipids). Encapsulation of β -carotene with semi-synthetic amphiphilic polymers has lead to cost effective slow release products with regulated solubility and enhanced bioavailability.
6. Most of the functional foods available in the market are usually colored with synthetic colorants which can be harmful to humans. The natural colorants described above have been used to develop value-added red guava nectar, lemon juice, mango panna, mango juice, grape juice, and other products namely aonla candy golden/purple bread, biscuits, cakes, muffins, as well as frozen products such as ice creams. A novel fusion product 'Capsitom' a salsa formulation comprising of red capsicum and red tomato has also been developed which has the benefit of both tomato and capsicum antioxidants.

7. Processes/ Products/Technologies Developed

(List partner-wise major Process/ Product/Technology developed & outcome in quantifiable terms)

S. No	(Process/Product/Technology Developed	Adoption/ Validation/ Commercialization	Responsible Partner
1	Production of anthocyanin enriched guava and lemon nectar (RTS) and mango pana. It showed improvement in color, taste and general appeal score	Commercialized	IARI

2	Anthocyanin concentrates from black carrot and Jamun	Commercialized	IARI
3	Chilli oleoresins	Commercialized	IARI
4	Capsanthin concentrate	Commercialized	IARI
5	Capsaicinoid concentrate	Commercialized	IARI
6	Lycopene concentrate from tomato	Commercialized	IARI
7	Steviol glycoside concentrate from <i>Stevia rebaudiana</i> leaves	Commercialized	IARI
8	Anthocyanin rich grape beverage	Validated	IARI
9	Anthocyanin rich Aonla flakes	Validated	IARI
10	Anthocyanin and lycopene rich dairy products	Validated	IARI
11	Nutraceutical enriched vegetable RTS	Validated	IARI
12	Carotenoid enriched Capsitom (Fusion product of tomato & capsicum)	Validated	IARI
13	Nutraceutical rich bread/ cake/ biscuits	Validated	IARI

8. Patents (Filed/Granted)

S. No.	Title of Patent	Inventor(s) (Name & Address)	Filed/Published/Granted (No./Date)	Responsible Partner
1	Anthocyanin rich functional products from black carrots and its process thereof	Kaur, C., Walia S. and Pal RK.	Indian patent application No. 3130/DEL/2012	Divisions of Agril. Chemicals and Post Harvest Technology, IARI
2	Amphiphilic polymers based slow release nano-formulations of β -carotene and method of preparation thereof	Kumar J., Shakil, N.A., Singh, B.B., Walia, S.	Indian patent application No. 3744/DEL/2012	Division of Agricultural Chemicals, IARI
3	Carotenoid rich composition and process of its preparation.	Kaur, C., Walia, S., Nath, P., Saha, S.	Indian patent application No. 909/DEL/2012	Divisions of Agricultural Chemicals and Post Harvest Technology, IARI
4	Three patent applications on production of Steviosides from <i>Stevia</i> , anthocaynin complex for enhanced bioavailability and Phycocyanins from <i>Spirulina</i> are under preparation			

9. Linkages and Collaborations

S. No.	Linkages developed (Name & Address of Organization)	Date/Period From-To	Responsible Partner	Purpose
1	Prof. Muralee G Nair Senior Associate to Dean, Michigan state University, USA	2008-till date	Dr Suresh Walia	Sharing of knowledge and few exchange visits done from both sides
2	Mr. Pradhan, National Research and Development Council (NRDC)	2008-till date	Dr Suresh Walia	For commercialization of technologies
3	Balaji Crop Care Hyderabad.	Feb 2008-December, 2012	Dr Suresh Walia	Consortium partner
4	Ozone biotech Faridabad	Feb 2008- December, 2012	Dr Suresh Walia	Consortium partner
5	Chordia foods Pvt. Limited, Pune	Feb 2008- December, 2012	Dr Suresh Walia	Consortium partner
6	Pratistha Industries Secunderabad, AP	July 1, 2013-till date	Dr Suresh Walia	Signed MoU with IARI for the developed technologies

10. Status on Environmental and Social Safeguard Framework

The project does not foresee any ecological and environmental degradation as it deals with products and technologies derived from vegetables, fruits and other non-food crops. Given the present status of nutraceuticals and food industry and the activities of the sub-project, no adverse effect on environmental and social safeguards is anticipated. It will not have any adverse effect on natural habitat, cultural property, involuntary resettlement, indigenous peoples, forests and waterways etc. The processes/ techniques used in extraction or processing of vegetables/ fruits is not likely to generate undesired liquid or solid wastes of un-compostable nature that may require specific pre-treatments. Technically the sub-project aims to increase nutraceutical production through use of improved varieties and extraction/ processing technologies by adopting modern scientific tools. Necessary treatment facilities may however be required to treat non-hazardous industrial wastes that may come out during food processing.

11. Constraints, if any and Remedial Measures Taken

There was no major constraint observed during implementation of the project. One of the collaborating partner from the industry namely Agri Food Park Limited, Pune did not actively participated in the project and therefore funds sanctioned for the year 2011 (HY ending Dec 2011) and 2012 were not released by PIU-NAIP. This however, did not hamper the overall progress of the project.

12. Publications

A. Research papers in peer reviewed journals

S. No.	Authors, Title of the paper, Name of Journal, Year, Vol. & Page No.	NAAS Rating (2012)	Responsible Partner
1	Saha S, Walia S, Sharma K, Kundu A and Paul R K. 2014. Optimal extraction and fingerprinting of carotenoids by accelerated solvent extraction and liquid chromatography with tandem mass spectrometry. <i>Food Chemistry</i> (Accepted and in Press)	8.0	Agril Chemicals Division (IARI)
2	Saha S, Walia S, Kundu A, Kaur C and Sisodia R. 2014. Capsaicinoids, tocopherol and sterols content in chili (<i>Capsicum</i> sp.) by Gas chromatographic-mass spectrometric determination. <i>International Journal of Food Properties</i> . (Accepted and in Press)	7.1	Agril Chemicals Division (IARI)
3	Kaur C, Walia S, Nagal S, Walia S, Singh J, Singh B, Saha S, Singh B., Kalia, P., Jaggi S and Sarika. 2013. Functional quality and antioxidant composition of selected tomato (<i>Solanum lycopersicon</i> L) cultivars grown in northern India. <i>LWT - Food Science and Technology</i> . 50 : 139–145	7.7	Post Harvest Technology and Division of Agril Chemicals (IARI)
4	Raina R, Bhandari S K, Romesh Chand and Sharma Y P. 2013. Causes of poor seed germination in <i>Stevia rebaudiana</i> , a low calorie sweetener. <i>Journal of Medicinal Plant Research</i> 7 : 1793-1799	6.0	Dr. YS Parmar University of Horticulture and Forestry, Solan
5	Kumar D, Kumar N, Pabbi S, Walia S, Dhar D W. 2013. Protocol optimization for enhanced production of natural colors in <i>Spirulina</i> . <i>Indian Journal of Plant Physiology</i> 18 : 308-312	5.5	CCBGUA, IARI, New Delhi
6	Khandare V, Walia S., Singh M, and Kaur C. 2011. Black carrot (<i>Daucus carota</i>) sub-sp. <i>sativus</i> juice: Processing effects on antioxidant composition and color <i>Food and Bio-products Processing</i> 89 : 482-86	7.6	Post Harvest Technology, IARI, New Delhi
7	Walia S, Singh M, Kaur C., Kumar R and Subodh J. 2010. Antioxidant composition of red and orange cultivars of tomatoes (<i>Solanum lycopersicon</i> L): A comparative evaluation <i>Journal of Plant Biochemistry & Biotechnology</i> 19 : 95-97	7.0	Post Harvest Technology, IARI, New Delhi
Communicated			
8	Kaur C, Singh B, Walia S, Walia Shweta, Nagal S, Singh J., Singhal P, Jaggi S. and Sarika. 2013. Antioxidant	8.2	Post Harvest Technology and Division of Agril

	activity, total phenolics and <i>in-vitro</i> inhibitory potential of key enzymes relevant for type 2 diabetes in sweet peppers (<i>Capsicum annuum</i> L.) <i>Bioresource Technology</i> (Communicated)		Chemicals (IARI)
9	Kumar D, Kumar N, Pabbi S, Dhar D W and Walia S. 2013. Similarity analysis of <i>Spirulina</i> strains on the basis of Phycocyanin operon locus (cpcB-IGS-cpcA) and 16S rRNA gene sequences. <i>Journal of Microbiology</i> (Communicated)	7.5	CCBGUA, IARI
10	Kumar D, Kumar N, Pabbi S, Dhar D W and Walia S. 2013. Extraction and purification of C-Phycocyanin from <i>Spirulina platensis</i> (CCC540). <i>Journal of Microbiology</i> (Communicated)	7.5	CCBGUA, IARI

B. Books/ Book chapters/ Abstracts/ Popular articles, Brochures, etc.

S. No	Authors, Title of the papers Name of Book/ Seminar/ Proceedings/Journal, Publisher, Year, Page No.	Responsible Partner
Book Chapters/ Proceedings papers		
1	Walia S. 2009. New innovations in extraction, value addition and quality Control of Nutraceuticals from High Value Vegetables, Fruits and Microalgae. (in) Lead papers: 9 th National Agricultural Science Congress, Srinagar (June 22-24, 2009), National Academy of Agricultural Sciences, New Delhi, p 316-322.	IARI, New Delhi
2	Walia S. 2012. Bioactive natural products as crop protectants, medicines and health benefitting nutraceuticals. (in) Proceedings, National Seminar on 'New vistas in aromatic crops-priorities and research accomplishments' Horticulture Society of South India, TNAU Kodaikanal. p 152-158.	IARI, New Delhi
3	Walia S. 2013. Extraction of bio-active compounds from horticultural crops: Emerging technologies. (in) <i>Horticulture for Food and Environment security</i> , p 459-472, Chadha K L, Singh A K, Singh S K and Dhilon W S (Eds), Westville Publishing House New Delhi	Division of Agril. Chemicals, I.A.R.I., New Delhi
Abstracts		
1	Walia S. (2009) Vegetable and Fruit-derived Nutraceuticals: Extraction and Quality Control. International Conference on Food Technology, IICPT, Thanjavur, (August 28-30, 2009).	Division of Agril. Chemicals, I.A.R.I., New Delhi
2	Kumar D., Saxena S., Singh NK, Pabbi,S., Dhar DW and Walia S. (2009) Comparative studies amongst selected strains of <i>Spirulina/Arthrospira</i> . In Conference on "Third Golden Era of	CCBGUA (IARI)

	Microbiology”, Organized by Association of Microbiologists of India at National Chemical Laboratory and Department of Microbiology, University of Pune, December 15-18.	
3	Kumar D., Saxena S., Kumar N., Pabbi S., Walia S., Dhar DW. (2010) Protocol optimization for mass production of <i>Spirulina</i> . In A National Seminar on Microbiological Approaches to Environmental Sustainability Organized by Department of Microbiology and Food Science and Technology, GITAM institute of Science, GITAM UNIVERSITY, 21-22 December, 2010 Visakhapatnam AP.	CCBGUA IARI, New Delhi
4	Kumar, D., Dhar DW., Saxena S and Pabbi S. (2010) Screening and mass production of cyanobacterial genus <i>Spirulina</i> : A potential food supplement, Poster presented in National Conference of Plant Biodiversity and Energy Resources <i>vis a vis</i> Biotechnological Tools for Sustainability, Department of Botany, Chaudhary Charan Singh University, Meerut-250004; December 25-26,2010	CCBGUA IARI, New Delhi
5	Walia S (2010). <i>Baseline survey of the NAIP sub-project</i> “Value chain on production of food grade nutraceuticals for use as natural antioxidants and food colorant. (Report submitted to NAIP-PIU/ ICAR). pp 42	Division of Agril. Chemicals, I.A.R.I., New Delhi
6	Walia S (2010). <i>Case study on NAIP sub-projects</i> “Value chain on production of food grade nutraceuticals for use as natural antioxidants and food colorant. (Report submitted to NAIP-PIU/ ICAR). pp 27.	Division of Agril. Chemicals, I.A.R.I., New Delhi
7	Walia S (2010) New Innovations in Extraction, Value addition and Quality Control of Nutraceuticals from High Value Vegetables and Fruits. Invited lecture, Division of Vegetable Sciences, IARI, New Delhi (November 2, 2010).	Division of Agril. Chemicals, I.A.R.I., New Delhi
8	Walia S (2010) Extraction, separation and analysis of bioactive natural products Lecture delivered in the Training Programme conducted by CCUBGA, Division of Microbiology, IARI, New Delhi (May 16, 2010).	Division of Agril. Chemicals, I.A.R.I., New Delhi
9	Walia S (2011) Recent advances in extraction, separation and analysis of nutraceuticals and other bioactive natural products, NAIP National Training Course, NIAH, Bikaner Feb 14-27, 2011.	Division of Agril. Chemicals, I.A.R.I., New Delhi
11	Walia S (2011) Nano-technological interventions in production and use of nutraceuticals and functional foods Bikaner. NAIP National Training Course, NIAH, Bikaner Feb 14-27, 2011	Division of Agril. Chemicals, I.A.R.I., New Delhi
12	Walia S (2011) Nutraceutical potential of pomegranate, jamun and other arid zone fruits, NAIP National Training Course, NIAH, Bikaner Feb 14-27, 2011.	Division of Agril. Chemicals, I.A.R.I., New Delhi
13	Walia S (2011) Nutraceuticals from food and non-food crops,	Division of Agril.

	NAIP sponsored workshop on “Innovative solutions to production of nutraceuticals and functional foods” (March 22-26, 2011), Division of Agril. Chemicals, IARI, New Delhi.	Chemicals, I.A.R.I., New Delhi
14	Walia S (2011) Nutraceuticals in fermented foods: Production and scale up (December 9-24, 2011) NAIP sponsored National training on fermentation technology, Division of Post Harvest technology, IARI, New Delhi. March 14-27, 2011.	Division of Agril. Chemicals, I.A.R.I., New Delhi
15	Dhar D W, Kumar D, Saxena S, Pabbi S, and Walia S (2011) Screening and mass production of cyanobacterial genus <i>Spirulina</i> . Poster (P-079) presented in 4 th Congress of the International Society for Applied Phycology, June 19-24, 2011; at Halifax, Canada ; Organized by International Society for Applied Phycology and National Research Council, Canada.	CCBGUA (IARI)
16	Kumar D., Dhar DW, Saxena S, Walia S., and Pabbi S. (2012) Similarity analysis of <i>Spirulina</i> strains on the basis of Phycocyanin operon locus (cpcB-IGS-cpcA) and 16S rRNA gene sequences, Poster presented in International Conference on Biotechnology: A Rendezvous with Basic Science for Global Prosperity (BTBS-2012. NASC Complex IARI, PUSA, New Delhi; Dec. 25-27, 2012.	CCBGUA (IARI)
17	Sisodia S., Saha S., Kaur C., Walia S., (2012) Extraction and analysis of carotenoids and capsaicinoids in different chilli cultivars of Indian origin. 7 th Nutra India Summit held at Hotel Lalit Ashok, Bengaluru March 15-17, 2012 (Best poster award)	Division of Agril. Chemicals, I.A.R.I., New Delhi
18	Singh, J., Walia, S., Saha, S., Kaur, C., Kalia, P. (2012) Extraction and use of black carrot anthocyanins to enhance antioxidant activity and storage stability of mango and guava juice. 5 th Indian Horticulture congress, 6-9 th Nov. 2012. PAU, Ludhiana (Best poster award)	Division of Agril. Chemicals, I.A.R.I., New Delhi

13. Media Products Developed/Disseminated

S. No.	CD, Bulletins, Brochures, etc. (Year wise)	No. of Copies	Distribution	Responsible Partner
1	Walia S. 2011. Food grade nutraceuticals and functional foods from food and non food crops. IARI, New Delhi-110012 (5 minute video)	3	Submitted to PIU-NAIP	S. Walia
2	Walia S. 2011. CD-Nutraceutical production and use. Indian Agricultural Research Institute, New Delhi	10	10	S. Walia
3	Walia S. 2012. Health benefitting	-	-	S. Walia

	nutraceuticals from vegetables and fruits. A2Z TV programme show Krishi Samman. January 28, 2012 (broadcast two times)			
4	Walia S. 2012. Brochure – Value chain on Food Grade nutraceuticals for use as natural antioxidants and food colorants, IARI, N.Delhi	200	200	S. Walia
5	Walia S. 2011. Value chain on production of nutraceutical for use as natural antioxidant and food colorants. IARI, New Delhi (25 minute Video)	Video films of 5 minute duration (revised and updated)	Submitted to PIU-NAIP	S. Walia

14. Meetings/Seminars/Trainings/Kisan Mela, etc. organized

S. No.	Details of Meetings/Seminars/Trainings, etc.	Duration (From-To)	No. of Personnel Trained	Budget (lakhs)	Organizer (Name & Address)
Stakeholders/Scientists training					
1	Innovative Solutions to Production of nutraceuticals and functional Foods	22 nd March - 26 th March 2011	15	2.25	Dr S. Walia, Division of Agric. Chemicals , IARI, New Delhi
2	Naturally occurring nutraceuticals, crop protectants and other biomolecules for application in human and crop health	23 rd Jan-2 nd Feb 2012	22	5.30	Dr S. Walia, Division of Agric. Chemicals , IARI, New Delhi
3	Health benefitting phytochemicals from vegetables, fruits and non food crops	20 th -23 rd March 2012	10	1.95	Dr S. Walia, Division of Agric. Chemicals , IARI, New Delhi
4	Extraction and analysis of nutraceuticals from vegetables, fruits and non-food crops	22 nd -24 th Nov 2012	19	1.74	Dr S. Walia, Division of Agric. Chemicals , IARI, New Delhi

Farmers trainings

1	Farmers Training Camp	(March 16-3-2012)	30	0.50	Dr YS Parmar University of Horticulture and Forestry, Solan (HP)
2	Farmer's Training	26 th Dec 2012	40	0.50	Dr. Pritam Kalia, and Dr S. Walia, IARI, New Delhi.



Picture from left to right: Farmers training camp on March 16, 2012, distribution of stevia saplings to farmers, workshop during Oct 29-31, 2012, Farmers' field day on Dec 26, 2012

15. Participation in Conference/ Meetings/Trainings/ Radio talks, etc.

S. No .	Details of Meetings/Seminars/ Trainings/Radio talk, etc.(Name &Address)	Duration (From-To)	Budget	Participant (Name & Address)
1	9 th National Agricultural Science Congress at SKUAST Srinagar,	June 22-24 2009	NAIP	Walia, S. IARI, New Delhi

2	Third Golden Era of Microbiology, Organized by Association of Microbiologists of India at National Chemical Laboratory Pune	Dec. 15-18 2009	NAIP	Kumar D., Saxena, S. Singh N., Pabbi S, Dhar DW and Walia, S. IARI New Delhi
3	National Seminar on Microbiological Approaches to Environmental Sustainability at GITAM University, Visakhapatnam	Dec. 21-22 2010	NAIP	Kumar D, Saxena S, Kumar N., Pabbi S Walia, S and Dhar DW, IARI New Delhi
4	National Conference of Plant Biodiversity and Energy Resources vis a vis Biotechnological Tools for Sustainability at Chaudhary Charan Singh University, Meerut	Dec. 25-26, 2010	NAIP	Kumar D., Dhar DW, S. & Pabbi, S IARI New Delhi
5	Congress of the International Society for Applied Phycology, at Halifax, Canada; Organized by International Society for Applied Phycology and National Research Council	June 19-24, 2011	NAIP	Dhar D W, Kumar D, Saxena S, Pabbi S and Walia S, IARI New Delhi
6	6 th Nutraceutical Summit at CFTRI, Mysore,	Feb 15-18, 2011	NAIP	Walia S, Kaur Charanjit, Saha S, and Bhan C, IARI, N.Delhi
7	7 th Nutra Summit 2012 organised by CSIR and IUFOST,	March 15-17, 2012	NAIP	Saha S, Pathak N, Kundu A, Walia S.
8	7 th Nutra Summit 2012 organised by CSIR and IUFOST,	March 15-17, 2012	NAIP	Singh J, Saha S, Kaur C, Walia S.
9	Fifth Indian Horticulture Congress, PAU, Ludhiana	Nov. 6-9, 2012	NAIP	Singh J, Saha S, Kaur C, Kalia, P., Walia S.
10	National Science Congress in Indian Languages organized by Swadeshi Science Movement of India	Feb. 25-27, 2013	NAIP	Singh J, Walia S., Saha S, Kumar, B., Sharma, K, Kaur C, Kalia, P
11	Extraction and analysis of carotenoids and capsaicinoids in different chilli cultivars of Indian origin. 7 th Nutra India Summit held at Hotel Lalit Ashok, Bengaluru (Best poster award)	March 15-17, 2012	NAIP	Sisodia S., Saha S., Kaur C., Walia S.,
12	Nutraceutical profiling of different chilli varieties for major/minor capsaicinoids of their economic importance. 5 th Indian Horticulture Congress, PAU, Ludhiana.	6-9 th November. 2012	NAIP	Sisodia R., Walia S., Saha S., Kumar B., Kalia P.

TV talk			
1	Health benefitting nutraceuticals from vegetables and fruits. A2Z TV programme show Krishi Samman.	January 28, 2012 (broadcast two times)	S. Walia

16. Foreign Trainings/Visits

S. No.	Name, Designation & Address of the Person	Visit/Training/Seminar Organization and Duration (From-to)	Dates of Seminar Delivered & Report Submitted on Return	Follow up Action	Total Cost (lakhs)
1	Dr. Charanjit Kaur. Principal scientist	Training in Post-harvest technological advancements in fusion food production, Michigan State University, East Lansing, USA, 26 th March-25 th May 2010	Seminar: 13 th June 2010	Experience gained is being utilized for successful implementation of the project	5,20,861
2	Dr .N.A. Shakil, Senior scientist	Training in Nano-science application in nutraceuticals development, Institute of Nanoscience Engineering Technology, University of Massachusetts, USA, 21 st Jan-22 nd March 2010	Seminar: 3 rd April 2010	-do-	3,42,449
3	Dr Dolly Wattal Dhar, Principal scientist	Training in area of micro algal technology, Robe Leadership institute, Ohio State University, USA, 7 th Dec-4 th Feb 2010	Seminar: 2 nd March, 2010	-do-	3,22,238
4	Dr Suresh Walia, Principal scientist	Training in area of advance extraction technology for antioxidants and food colorants from food and non-food crops, National Food Safety & Toxicology Center, Michigan State University, East Lansing, 8 th Sept-26 th October 2010.	Seminar: Nov 23, 2010	-do-	4,16,367

17. Performance Indicators

S. No.	Indicator	Total No.	
1.	No. of production technologies released and/or adopted	9	
2.	No. of processing technologies released and/or adopted	9	
3.	Number of technologies/products commercialized based on NAIP research	6	
4.	No. of new rural industries/enterprises established/ upgraded	-	
5.	No. of product groups for which quality grades developed and agreed	2	
6.	Total no. of private sector organizations (including NGOs) participating in consortium	3	
7.	No. of farmers involved in consortia activities	50	
8.	Total number of farmers' group developed for marketing and processing	-	
9.	Number of patent/intellectual property protection applications filed based on NAIP research	3	
10.	Number of patents/intellectual property protections granted/published based on NAIP research	-	
11.	Number of scientists trained overseas in the frontier areas of science	-	
12.	Number of scientists trained overseas in consortium-based subject areas	4	
13.	No. of scientists participated in conference/seminar etc. abroad	2	
14.	Success stories	2	
15.	Incremental employment generated (person days/year/HH)	Baseline	Final
		-	-
16.	Increase in income of participating households (₹ per annum)	Baseline	Final
		-	-
17.	Number of novel tools/protocols/methodologies developed	6	
18.	Publications		
	Articles in NAAS rated journals	7 (Published) 3 (under review)	
	Articles in other journals	-	
	Book(s)	-	
	Book chapter(s)		
	Thesis	1	
	Popular article(s) (English)	-	
	Newspaper article(s)	2	
	Seminar/Symposium/Conference/Workshop Proceedings	4	
	Technical bulletin(s)	3	
	Manual(s)	3	
	CDs/Videos	2	
	Popular article(s) in other language	1	
	Folder/Leaflet/Handout	2	

Report(s)	4
Success stories (Technologies transferred to two industries)	2

18. Employment Generation (man-days/year)

S. No.	Type of Employment Generation	Employment Generation up to End of Sub-project	Responsible Partner
1.	Employment generation will be possible once the transferred technologies are commercialized by the industry and farmer's produce (vegetables, fruits and non-food crops) is procured and used as raw material.		
2.	Efforts will be made during 2011-12 to train farmers and encourage other stakeholders to take up value addition and processing of their agriculture produce in small/large scale		

19. Assets Generated

(Details to be given on equipments and works undertaken in the sub-project, costing more than ₹ 10,000/- in each case)

(i) Equipment

S. No.	Name of the Equipment with Manufacturer's Name, Model & Sr. no.	Year of purchase	Qty. (Nos.)	Total Cost (Rs.)	Responsible Consortium
1.	GC-MS with head space M/S Agilent Technologies, 7890A (GC) 5975C inert AL EL/CI MSD with Triple Axis Detector	2010 – 2011	01	58,85,650	Division of Agril. Chemicals
2.	HPLC system with DAD/ELSD/Fluorescence detector M/S Waters GES. MBH E-2695, 2998 (DAD), 2475 (Fluorescence), 2424 (ELSD)	2010 – 2011	01	35,92,220	Division of Agril. Chemicals
3.	HFC Extraction Unit M/S Thar SFC	2010 – 2011	01	30,16,043	Division of Agril. Chemicals
4.	Accelerated solvent extractor M/S Ameritech Scientific Cooperation, ASE- 350	2010 – 2011	01	29,31,180	Division of Agril. Chemicals

	Dionex				
5.	Deep Freezer -40°C Haier	2009 – 2010	01	1,80,375	Division of Agril. Chemicals
6.	UV-Vis spectrophotometer Varian	2009 – 2010	01	3,31,486	Division of Post Harvest technology
7.	Zetasizer Zetatrac, NPA 152 OM 0013, Metrohm	2009 – 2010	01	14,58,443	Division of Agril. Chemicals
8.	Ice Flaking machine Zeigra	2009 – 2010	01	3,65,256	Division of Agril. Chemicals
9.	Rotary evaporator (1 ltr. Capacity) Heidolph, Laborota 4001	2009 – 2010	02	6,75,896	Division of Agril. Chemicals
10.	Ultrasonicator Misonics Vibra m Cell	2009 – 2010	02	5,80,000	Division of Agril. Chemicals
11.	Flash Chromatography Isolera 1, Biotage	2009 – 2010	01	11,94, 045	Division of Agril. Chemicals
12.	Evaporation with auto control Genevac, Rocket	2009 – 2010	01	14,65,400	Division of Agril. Chemicals
13.	Spray Dryer Labutima	2009 – 2010	01	9,79,144	Division of Agril. Chemicals
14.	Water Purification system Millipore, Elix	2009 – 2010	01	3,72,084	Division of Agril. Chemicals
15	Rotavapour (5 L) Heidolph, Hei Vap Advantage	2009 – 2010	01	6,82,992	Division of Agril. Chemicals
16	Photobioreactor Labconco	2009 – 2010	01	19,03,280	Division of Microbiology

17	Lyophilizer (Tray Type) Labconco	2009 – 2010	01	11,43,667	Division of Agril. Chemicals
18	Homogeniser Ika, T-10 Basic Ultra-Turrax	2009 – 2010	01	1,09,725	Division of Agril. Chemicals
19	Autoclave Sanco	2009 – 2010	01	55,575	Division of Agril. Chemicals
20	Digital video camera Sony	2009 – 2010	01	49,500	Division of Agril. Chemicals
21	Laptop Sony, Vaio	2009 – 2010	01	83, 403	Division of Agril. Chemicals
22	Sonicator Sonics	2009 – 2010	01	2,84,854	Division of Agril. Chemicals
23	Rotavapor	2009 – 2010	01	2,59,656	Division of Agril. Chemicals
24	Incubator Shaker with light facility Labtech	2009 – 2010	01	2,57,875	Division of Agril. Chemicals
25	Deep Freezer Vestfrost	2009 – 2010	03	1,98,281	Division of Agril. Chemicals, Division of PHT, Division of Microbiology
26	Vacuum Dryer	2009 – 2010	01	4,99,980	Division of Agril. Chemicals
27	Freezer with glass door Celfrost	2009 – 2010	01	48, 091	Division of Agril. Chemicals
28	Refrigerator Samsung	2009 – 2010	03	62, 687	Division of Agril. Chemicals, Division of PHT, Division of Microbiology
29	Filling and Sealing machine	2009 – 2010	01	1,99,680	Division of Post Harvest technology

	Indus				
30	Hydraulic Press Unitech	2009 – 2010	01	2,24,640	Division of Post Harvest technology
31	Air conditioners Voltas	2009 – 2010	04	1, 38, 350	Division of Agril. Chemicals
32	Laminar Flow Labtech	2009 – 2010	01	2,41,875	Division of Agril. Chemicals
33	Electronic Balance Sartorius	2009 – 2010	01	1,74,150	Division of Agril. Chemicals
34	Incubator shaker without light facility	2009 – 2010	01	2,74,800	Division of Agril. Chemicals
35	Rotavapour, Heidolph	2009-2010	01	3,50,000	Dr YS Parmar University of Horticulture and Forestry, Solan (HP)
36	Spray dryer, Labutima	2009-2010	01	10,00,000	Dr YS Parmar University of Horticulture and Forestry, Solan (HP)
37	Electronic balance, Sartorius	2009-2010	01	1,50,000	Dr YS Parmar University of Horticulture and Forestry, Solan (HP)
38	Deep freeze, Celfrost	2009-2010	01	75,000	Dr YS Parmar University of Horticulture and Forestry, Solan (HP)
39	Refrigerator, Samsung	2009-2010	01	25,000	Dr YS Parmar University of Horticulture and Forestry, Solan (HP)
40.	Industrial Centrifuge, Axiva Sichem Biotek	2010-11	01	60,900	IARI
41.	BOD Incunbator, Ferrotek	2010-11	01	75,600	IARI
42.	Liquid Nitrogen Containers, Reliable Instruments	2010-11	02	1,53,000	IARI
43.	UPS System,	2010-11	01	2,07,900	IARI

	Galaxy Scientific, Apex 11034021				
44.	Algal Harvester, Axiva SicheM Biotek	2010-11	01	1,71,100	IARI

(ii) Works

S. No.	Particulars of the Work, Name and Address of Agency Awarded the Work	Year of Work Done	Quantity (Nos.)	Total Cost (Lakhs)	Responsible Partner
1.	Nutraceutical laboratory, Sanjeev Construction and Engineering, S-16 West Patel Nagar, New Delhi-11008	2010-11	01	13.69	Division of Agric. Chemicals, IARI
2.	Analytical laboratory, Narender Construction co.C-149, Moti Nagar, New Delhi 110015	2010-11	01	10.61	Division of Agric. Chemicals, IARI
3	Repair and upgradation of algal production facility, ozone biotech, New Delhi.	2010-2011	01	3.548	Ozone Biotech, New Delhi
4	Renovation of post harvest laboratory, Narender Construction co.C-149, Moti Nagar, New Delhi-15	2009-2010	01	0.979	IARI
5	Renovation of algal laboratory, Narender Construction co.C-149, Moti Nagar, New Delhi-15	2010-2011	01	2.186	IARI

(iii) Revenue Generated

(Details may be given on revenue generated in the sub-project viz., sale of seeds, farm produce, products, patents, commercialization, training, etc.)

S. No.	Source of Revenue	Year	Total amount (Rs. lakh)	Responsible Partner
1	Memorandum of Understanding (MOU) has been signed/to be signed with M/s Ozone Biotech Faridabad for transfer of	2011	7.50	Suresh Walia and the Sub-Project team

	technology for nutraceuticals and functional foods developed under the project			
2	Memorandum of Understanding (MOU) has been signed with M/s Pratistha Industries Hyderabad for transfer of technology for nutraceuticals and functional foods developed under the project	2013	8.00	Suresh Walia and the Sub-Project team
TOTAL			15.5 lakh	

(iv) Livestock (Details of livestock procured/produced in the sub-project)

S. No.	Details of Livestock (Breed, etc.)	Year of Procurement/Production	Nos.	Total Cost (Rs)	Responsible Partner
NA					

20. Awards and Recognitions

S. No.	Name, Designation, Address of the Person	Award/ Recognition (with Date)	Institution/ Society Facilitating (Name & Address)	Responsible Partner
1	Sisodia S., Saha S., Kaur C., Walia S.,	Best poster award	7 th Nutra India Summit held at Hotel Lalit Ashok, Bengaluru March 15-17, 2012	IARI, New Delhi
2	Singh, J., Walia, S., Saha, S., Kaur, C., Kalia, P. (2012)	Best poster award	5 th Indian Horticulture congress, 6-9 th Nov. 2012. PAU, Ludhiana	IARI, New Delhi

21. Steps Undertaken for Post NAIP Sustainability

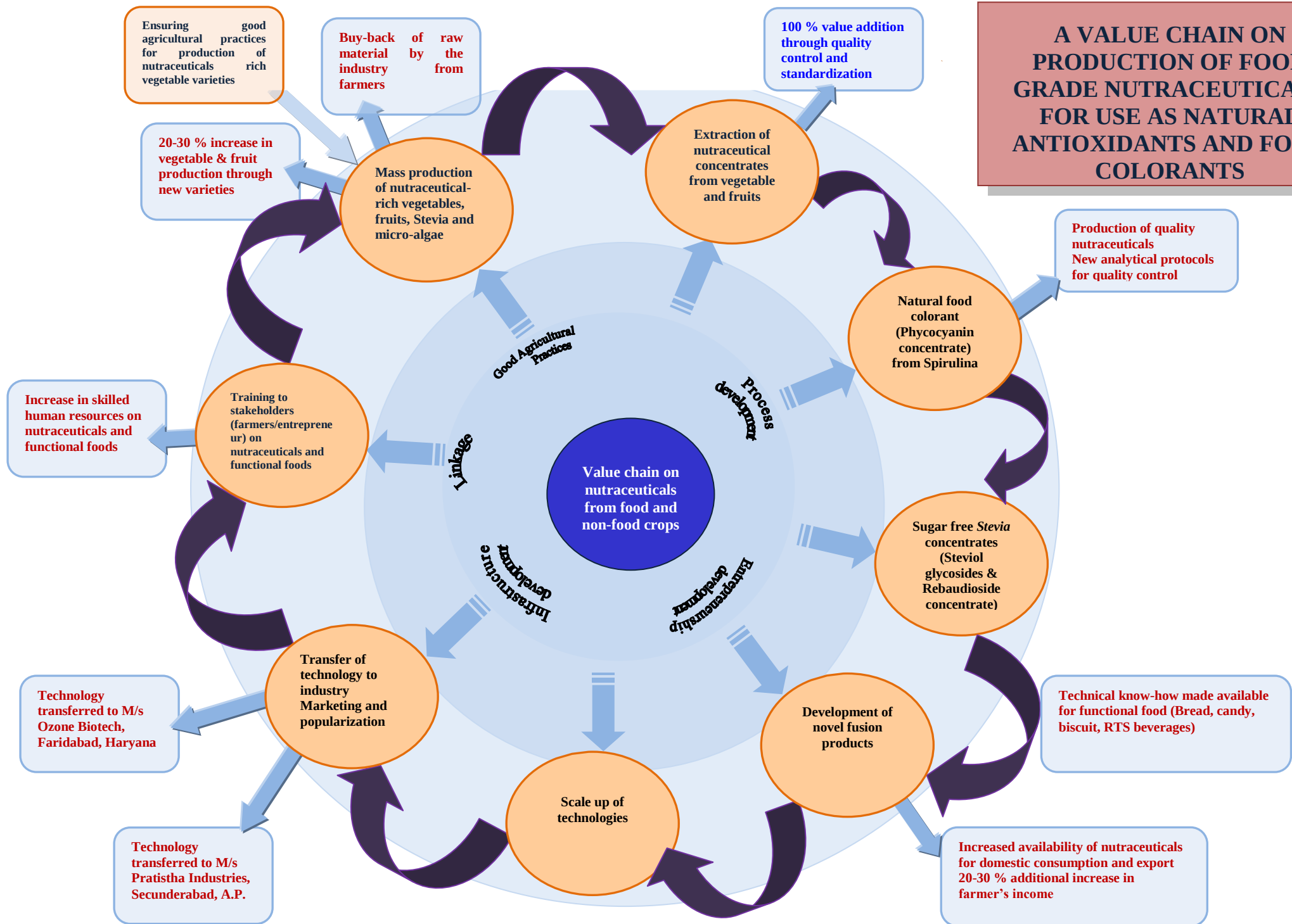
For post project sustainability, technical know-how developed for various value added products under the project will be transferred to industry /interested stakeholders for possible commercialization.

- Training/demonstration to potential stakeholders
Training will continue to be imparted to farmers to improve cultivation of potential nutraceuticals rich vegetables for better return. Stakeholders other than farmers (industry, entrepreneurs) be organized from time to time potential nutraceuticals rich vegetables
- Promotion of cultivation of potential nutraceuticals rich vegetables

Value-chain for production of food grade nutraceuticals for use as natural antioxidants and food colorants

Issues	Reasons	Interventions	Outcome	Impact	Sustainability
Non-utilization of vegetables, fruits and non-food crops for nutraceutical production	Lack of awareness about nutraceuticals as health benefitting compounds Raw material non-availability	Production of nutraceutical-rich vegetables, fruits, microalgae and stevia plantation through by ensuring good agricultural and laboratory practices	Identified varieties (tomato, chilli, black carrot) with rich nutraceutical content	Increased production of nutraceuticals rich vegetables will have positive impact on farmer's income	Continuing promotion of cultivation of nutraceutical rich vegetable varieties
Lack of focused intervention on production of phytochemicals from food and non food crops	Non-availability of process/technologies for production of value added nutraceuticals	Extraction of phytochemicals from black carrot, capsicum, tomato, jamun and non-food crop, Stevia and Spirulina	Vegetable and fruit concentrates rich in nutraceutical content	Other industries can use nutraceuticals concentrate in production of functional foods	Continuing development of know-how for production of nutraceuticals from unexplored crops
Lack of functional foods enriched with nutraceuticals (natural food colorants)	Non-availability of cost effective indigenous technology for the production of functional foods	Development of novel fusion products/functional foods	Availability of new analytical protocols for food grade phytochemicals	Standard and stable nutraceuticals products will have greater acceptability	Continue developing nutraceuticals based functional products
Lack of standard analytical protocols for analysis and quality control	Absence of proper research and analytical infrastructure	Development of standard protocols for nutraceutical analysis (HPLC, LC-MS, GC-MS) in finished products	Innovative functional foods (Aonla candy, mango panna, guava nectars, naturally coloured bread/biscuit) products for commercialization	Greater availability of health benefitting nutraceuticals to the end users	Research infrastructure will be available to others for analysis and quality control
		Creating trained/skilled human resource & infrastructure for capacity building and entrepreneurship development	Skilled human resource	Nutraceutical industry will be benefitted & more industries will be willing to enter into the business	Continue developing the human resources through training by all the consortium partners
Lack of entrepreneurship in development and use of nutraceuticals	Potential of nutraceuticals not appreciated by industry	Scale up and transfer of technology to the entrepreneurs for commercialization, income and employment generation	MoU signed with two industries for technology transfer	Production of nutraceuticals and functional foods to start after industry set up	Continue developing linkages with more potential entrepreneurs

A VALUE CHAIN ON PRODUCTION OF FOOD GRADE NUTRACEUTICALS FOR USE AS NATURAL ANTIOXIDANTS AND FOOD COLORANTS



- Transfer of technology to industry

MOU with following two companies have been signed for transfer of technology developed under this NAIP sub-project.

1. M/s Ozone Biotech Pvt. Ltd., Faridabad (Haryana)	Rs 7.5 lakh) (non-exclusive)
2. M/s Pratistha Industries, Secunderabad (AP)	Rs 8.0 lakh (non-exclusive)

Since the technology has been transferred to industry on non-exclusive basis more entrepreneurs can forward and enter into the expanding food industry basis. The above necessary steps will help the industry, farmers and the stakeholders for successful commercialization of any technology in the future

22. Possible Future Line of Work (Comments/suggestions of CPI regarding possible future line of work that may be taken up arising out of this sub-project)

Following are some of the future line of work arising out of this work which may be taken up by the ICAR scientists in future

- Develop technical know-how for cost-effective production of value-added nutraceuticals from unexplored vegetables, fruits, microalgae, marine products, bacteris, and fungi
- Exploring potential of under-utilized fruit and vegetable crops for their natural food colorants and antioxidants. Some potential unexplored/undrer-utilized fruits of arid/semi-arid region such as *Capparis decidua* (Ker) Capparidaceae family, *Diospyros melanoxylon* (Tendu) (Ebenaceae family), *Grewia subinaequalis* (Phalsa) (Tiliaceae family) , *Manilkara hexandra* (Khirmi) Sapotaceae family, *Salvadora* Species (Pilu and Miswak, *Ziziphus* Species (Ber) Rhamnaceae family, *Cucumis callous* (Kachri) Cucurbitaceous famil , *Aegle marmelos* (Bael)*Garcinia indica* (Kokum) have great future and needs to be explored for their nutraceutical potential.
- Deveopment of novel extraction technology utilizing modern tools and safer solvents such as water for efficient extraction and recovery of polyphenols/anthocyanins
- To evelop standard protocols for the analysis of antioxidants, food colorants and fusion products enriched with such nutraceuticals
- Finding innovative solutions to overcome the issue of inadequate thermal and photo-stability of nutraceutical ingredients and functional foods enriched with them
- Development of certified reference materials/ analytical procedures
- Nanotecnological intervention for enhancement of functional attributes of nutraceuticals and allied products.
- Application of nanotechnology in processing, value addition and packaging of nutraceutical products.
- Development of micro/ nanoencapsulated and nanosized smart delivery system for improved utilization of nutraceuticals in humans

23. Personnel

(Staff of Lead Centre & Partner-wise, their Name, Designation, Discipline and Duration)

Research Management (CL)	From – To (DD/MM/YYYY)
1. Dr.H.S.Gupta, Director, IARI, New Delhi Consortium Leader	13/02/2009-31/12/2012
2. Dr Malavika Dadlani, Joint Director (Res) Chairperson, CIC	13/02/2009-31/12/2012
Scientific (CPI, CCPI, others)	
3. Dr Suresh Walia, Division of Agric. Chemicals , IARI, New Delhi	13/02/2009-31/12/2012
4. Dr Jitendra Kumar, Division of Agric. Chemicals , IARI, New Delhi	13/02/2009-31/12/2012
5. Dr N.A. Shakil, Division of Agric. Chemicals , IARI, New Delhi	13/02/2009-31/12/2012
6. Dr. Supradip Saha, Division of Agricultural Chemicals , IARI, New Delhi	30/01/2010-31/12/2012
7. Dr Dolly Wattal Dhar, Division of Microbiology, IARI, New Delhi	13/02/2009-31/12/2012
8. Dr Sunil Pabbi, Division of Microbiology, IARI, New Delhi	13/02/2009-31/12/2012
9. Dr Pritam Kalia, Division of Vegetables, IARI, New Delhi.	13/02/2009-31/12/2012
10. Dr. Charanjit Kaur, Division of Post harvest Technology,IARI, New Delhi	13/02/2009-31/12/2012
11. Dr. Ramesh Rana, Department of Forest Products, Dr YS Parmar University of Horticulture and Forestry, Solan (HP)	13/02/2009-31/12/2012
12. Dr Ravinder Raina, Department of Breeding, Dr YS Parmar University of Horticulture and Forestry, Solan (HP)	13/02/2009-31/12/2012
13. Mr Pradeep Chordia, Managing Director, Agri Food Park Ltd. Pune	13/02/2009-30/06/2011
14. Mr Dhruv Gupta, Managing Director, Ozone Biotech Limited, Faridabad	13/02/2009-31/12/2012
15. Mr. R.L. Narayana Rao, Managing Director, Balaji Crop Care Pvt Limited Hyderabad	13/02/2009-31/12/2012
Technical	
NA	NA
Contractual	
16. Dr. Paraj Shukla	01/04/09-29/10/2009

17.	Mr. Ravi Prakash Pandey	01/04/09-19/07/10
18.	Mr. Jasbir Singh	01/04/09-31/12/2012
19.	Mr. Dhan Jai	01/04/09- 12/02/10
20.	Ms. Minakshi	01/04/09-1/05/10
21.	Mr. Devendra Kumar	01/04/09-31/12/2012
22.	Ms. Sharmila Ray	08/01/2010- 15/01/2011
23.	Ms Ritu Sisodia	10/06/10-31/12/2012
24.	Ms. Prerna Nath	10/06/10 -31/07/10
25.	Ms. Manveen Kaur Sethi	17/01/11-30/04/11
26.	Mr. Amit.K.Dixit	2/05/2011-31/10/2011
27.	Ms.Shweta Nagal	1/02/2011-31/03/2012
28.	Mr. Braj Bhusan Singh	19/01/11- 31/03/2012
29.	Mr. Rohit Bhatia	05/01/2012-31/12/2012

24. Governance, Management, Implementation and Coordination

A. Composition of the various committees (CIC, CAC, CMU, etc.)

S. No.	Committee Name	Chairman (From-To)	Members (From-To)
1.	CIC	Prof. K.R. Koundal, Joint Director (Res.), IARI New Delhi and Chairperson CIC (Feb.2009-Dec 2010)	Dr Dolly Wattal Dhar, Co-PI, CCBGU, Microbiology, IARI.(2009-2012)
		Dr. Malavika Dadlani, Joint Director (R) IARI New Delhi and Chairperson CIC (Jan 2011-Dec 2012)	Dr Charanjeet Kaur, PHT, Co-PI, IARI, New Delhi. (2009-2012)
			Dr. Romesh Rana, CCPI, Dr YSPUHF, Solan (H.P.) (2009-2012)
			Mr. Pradeep Chordia, CCPI, AgriFood parks Ltd., Pune. (2009-2012)
			Mr. Dhruv Gupta, CCPI, Ozone Biotech Ltd. Faridabad. (2009-2012)
			Mr. R.L. Narayana Rao, CCPI, Balaji Crop Care, Hyderabad. (2009-2012)
			Mr. Ravi Kumar, SAO, IARI
			Mr. G.D. Dubey, AF&AO, IARI
			Mrs. Poonam Singh, AAO, Div. of Agri. Chemicals. (2009-2012)
			Mr.T.S. Bhati AF&AO, IARI
			Mr.R.L.Pahija , SAO, IARI

		Mr. Radhey Sham Comptroller, IARI Dr Romesh Rana, Dept. of Forest Products, Dr YSPUHF, Solan. (2009-2012) Dr Ravinder Raina, Dept. of Breeding, Dr YSPUHF, Nauni, Solan. (2009-2012) Dr Jitendra Kumar, Division of Agricultural Chemicals. (2009-2012) Dr N.A. Shakil, Division of Agricultural Chemicals (2009-2012) Dr. Supradip Saha, Division of Agricultural Chemicals (2010-2012) Dr .Sunil Pabbi, CCUBGA, Division of Microbiology. (2009-2012) Dr Pritam Kalia, Division of Vegetables.(2009-2012)
2. CAC	Dr. Siva Kumar Chairman (Feb. 2009-Dec. 12)	Dr. Amir Azam (2009-2012), Professor, Jamia Milia Islamia University, New Delhi, and Member CAC
		Dr. D.S. Khurdiya, Ex. Head, PHT, IARI, New Delhi. (2009-2012), and Member CAC Dr. S.M. Gupta, Associate Director, Ranbaxy Laboratories, R & D, Gurgaon, and Member CAC Dr. J.P. Mittal, NC (Component II), NAIP- PIU.(2009-2009) Dr. R.K. Goyal, NC (Component II), NAIP- PIU. (2009-2012) Dr. K.R. Koundal, Joint Director (Research), IARI New Delhi Dr.Malavika Dadlani, Joint Director (Research), IARI New Delhi Dr. Suresh Walia, Div. of Agril. Chemicals, IARI (2009-2012)
3. CMU	Dr. H. S. Gupta, Director, IARI, & Consortium Leader (Feb. 2009-Dec. 2012)	Prof. K.R. Koundal, Joint Director (Research), IARI New Delhi (2009-2012) Dr. Suresh Walia, CPI, Div. of Agril. Chemicals, IARI(2009-2012) Dr Dolly Wattal Dhar, Co-PI, CCBGU, Microbiology, IARI. (2009-2012) Dr Charanjeet Kaur, PHT, Co-PI, IARI, New Delhi. (2009-2012) Dr. Romesh Rana, CCPI, Dr YSPUHF, Solan (H.P.). (2009-2012)

	Dr Jitendra Kumar, Division of Agricultural Chemicals. (2009-2012)
	Dr N.A. Shakil, Division of Agricultural Chemicals. (2009-2012)
	Dr. Sunil Pabbi, CCUBGA, Division of Microbiology. (2009-2012)
	Dr Pritam Kalia, Division of Vegetables. (2009-2012)
	Dr. Attril, Div. Of Agril. Economics, IARI, New Delhi

A. List of Meetings organized (CIC, CAC, CMU, etc.)

S. No.	Details of the meeting	Date	Place & Address (Where meeting was organized)
1. CIC		18 th May 2009	Division of Agricultural Chemicals, IARI, New Delhi
		24 th Nov 2009	Division of Agricultural Chemicals, IARI, New Delhi
		29 th Jan 2010	Division of Agricultural Chemicals, IARI, New Delhi
		16 th July 2010	Division of Agricultural Chemicals, IARI, New Delhi
		8 th Feb 2011	Division of Agricultural Chemicals, IARI, New Delhi
		24 th Dec 2011	Division of Agricultural Chemicals, IARI, New Delhi
		19 th March 2012	Division of Agricultural Chemicals, IARI, New Delhi
2. CAC		19 th May 2009	Division of Agricultural Chemicals, IARI, New Delhi
		25 th Nov 2009	Division of Agricultural Chemicals, IARI, New Delhi
		30 th Jan 2010	Division of Agricultural Chemicals, IARI, New Delhi
		9 th March 2011	Dr.Y.S.Parmar, U.H.& F, Solan (HP)
		24 th Dec 2011	Division of Agricultural Chemicals, IARI, New Delhi
		19 th March 2012	Division of Agricultural Chemicals, IARI, New Delhi
		28 th Dec 2012	Division of Agricultural Chemicals, IARI, New Delhi
3. CMU		30 th Jan 2010	Review meeting under the Chairmanship of Director, IARI in the Division of Agril. Chemicals, IARI, New Delhi
		11 th March 2011	Review meeting under the Chairmanship of Director, IARI
		12 th Sept., 2011	Review meeting under the Chairmanship of Director, IARI (Financial progress and procurement of equipments)
		13 th Jan. 2012	Review meeting under the Chairmanship of the Director IARI
		4 th Feb. 2012	Review Meeting with the Comptroller, IARI regarding progress of expenditure
		4-5 May, 2012	Review meeting under the Chairmanship of ND (NAIP) and presided by Director, IARI, and Chairman CAC & CIC
		6 th Oct. 2012	Review meeting under the Chairmanship of the Director IARI in the Board Room, Director's office

Part-III: Budget and its Utilization

STATEMENT OF EXPENDITURE (Final) (Period from 13th Feb 2009 to 30th December 2012)

Sanction Letter No.	F.No.1 (5)/2007-NAIP
Total Sub-project Cost	Rs 629.6962
Sanctioned/Revised Sub-project cost (<i>if applicable</i>) `	Rs 639.8665
Date of Commencement of Sub-project:	13 th Feb 2009
Duration:	From 13 th Feb 2009 to 31 st Dec 2012
<u>Funds allocated in each year (in lakhs)</u>	
I Year (Feb. 13-March 31, 2009)	Rs. 3.3423/-
II Year (FY 2009-2010)	Rs. 230.6857/-
III Year (FY 2010-2011)	Rs. 259.4831/-
IV year (FY 2011-2012)	Rs. 86.3581/-
V year (April 2012- Dec. 2012)	Rs. 56.9973/-
Bank Interest received on funds (<i>if any</i>) Rs 34,520	
Total amount received	Rs. 636.8665/-
Total expenditure	Rs. 628.13824/-

Expenditure Head-wise:

Sanctioned Heads	Funds Allocated (*)	Funds Allocated					Expenditure Incurred					Total Expend	Balance as on date
		1 st Yr	2 nd Yr	3 rd Yr	4 th Yr	5 th Yr	1 st Yr	2 nd Yr	3 rd Yr	4 th Yr	5 th Yr		
A. Recurring Contingencies													
1. TA	12.1222	0.1705	2.989	3.7342	2.87877	2.35	0.1705	2.989	3.73425	2.37877	0.8641	10.13662	1.98558
2. Workshops	7.63111	0	0.53	2.3456	2.0055	2.75	0	0.53	2.34561	2.0054	1.99995	6.88096	0.75015
3. Contractual Services RA/SRF	57.71669	0	11.74723	14.7337	20.38775	10.848	0	11.7472	14.73371	20.38775	9.45473	56.32342	1.39327
4. Operational costs	163.4944	3.1658	39.3296	42.8819	43.1470	34.97	3.16583	39.3196	42.88196	43.64706	34.06559	163.08013	0.41427
Sub-Total of A (1-4)	240.9647	3.3363	54.5959	63.6955	68.4190	50.918	3.33633	54.5959	63.69553	68.41897	46.38437	236.43112	4.53358
B. HRD Component													
5. Intl. Training	16.01041	0	11.9398	4.07061	0	0	0	11.93987	4.07061	0	0	16.01048	-0.00007
6.National.training	1.5	0	0	0	0	1.5	0	0	0	0	0.96	0.96	0.54
(7) Consultancy	0	0	0	0	0	0	0	0	0	0	0	0	0
Sub-Total of B (5-7)	17.5104	0	11.9398	4.07061	0	1.5	0	11.93987	4.07061	0	0.96	16.97048	0.53992
C. Non-Recurring													
(7) Equipment	328.1047	0	158.4606	160.09745	9.546739	0	0	158.4606	160.09745	9.546739	0	328.10483	-0.00013
(8) Furniture	0.49823	0	0.49823	0	0	0	0	0.49823	0	0	0	0.49823	0
(9) Works (new renovation)	34.0894	0	3.9660	26.6616	3.46183	0	0	3.96608	26.66164	3.36479	0	33.99251	0.09689
(10) Others (Animals, Books, etc.)	0.1333	0	0.0	0	0.1333	0	0	0	0	0.13338	0	0.13338	-0.00008
Sub-Total of C (7-10)	362.8258	0	162.9249	254.525	13.1419	52.418	0	162.9249	186.75909	13.044909	0	362.72890	0.0969
D. Institutional Charges*	15.5651	0.006	1.225	4.95791	4.7970	4.5793	0.006	1.225	4.95791	4.79709	1.02174	12.00774	3.55736
Grand Total (A+B+C+D)	636.8665	3.3423	230.6857	259.483	73.31903	56.9973	3.34233	230.6857	259.48314	86.260969	48.36611	628.13824	8.73724

1st Yr: 2008-2009; 2nd Yr: 2009-2010; 3rd Yr: 2010-2011; 4th Yr: 2011-2012; 5th Yr: April 2012, -Dec, 2012

Name & Signature of CPI :



PART-IV: DECLARATION

This is to certify that the final report of the Sub-project has been submitted in full consultation with the consortium partners in accordance with the approved objectives and technical programme and the relevant records, note books; materials are available for the same.

Place: New Delhi

Date: 11.02.2014



Signature of Consortium Principal Investigator



Signature

Consortium Co-Principal Investigator

(Dr. Ramesh Rana)



Signature

Consortium Co-Principal Investigator

(Mr. Dhruv Gupta)



Signature

Consortium Co-Principal Investigator

(Mr. R.L.Narayana Rao)

It was one of the most successful consortium as evidenced by the output.



Comments & Signature of Consortium Leader

Dr.H.S.Gupta, Director, IARI, New Delhi

Dr. H. S. Gupta

Director

Indian Agricultural Research Institute
New Delhi - 110012 (India)

DETAILS OF TECHNOLOGIES/INNOVATIONS DEVELOPED & COMMERCIALIZED

Value chain for the production of food grade nutraceuticals for use as natural antioxidants and food colorants

CPI: Dr. Suresh Walia

- 1) Title of the technology: *Extraction and production of anthocyanin concentrate from black carrot and jamun*
- 2) Commercialization status with date of licensing/ MOU:
Memorandum of Understanding (MOU) has been signed with M/s Ozone Biotech, Faridabad on March, 2011 and M/s Pratistha Industries, Hyderabad on July 18, 2013 for transfer of technology
- 3) Brief description of intervention/ innovation:
Synthetic food colors are no longer an attractive option as global markets are driving towards clean and natural colours. Black carrot is a rich source of natural stable acylated anthocyanins which impart black carrots their dark violet color. Jamun is also an excellent source of anthocyanins. Such anthocyanins provide health benefits due to their significant antioxidant activity and their ability to promote immune systems. Bench scale know-how has been developed for the extraction of anthocyanins from jamun and black carrot juice and/or concentrate. Anthocyanin concentrate has been further purified to obtain crystalline anthocyanin powder which can be used as a potential source of natural food colorant. The concentrates can be used to prepare nutraceutical enriched processed foods, beverages (fruit drinks, alcoholic drinks), jams, candy, pro-biotics, and frozen foods like ice creams/ yogurts by imparting pleasing pink/ purple colour. The process is simple, cost effective and involves food grade solvents.
- 4) Name and address of the firm(s) which has commercialized it:
M/s Ozone Biotech, Faridabad on March 3, 2011
M/s Pratistha Industries, Hyderabad on July 18, 2013
- 5) Area (state(s)/ district(s)) covered:
M/s Ozone Biotech, Faridabad will cover Northern India
M/s Pratistha Industries, Hyderabad will cover Southern India
- 6) Volume/ quantity of Annual production and approximate sale value: NA
- 7) Benchmark (existing similar product) and Consumer acceptance, particularly in case of food products:
No such similar products are present in the Indian market.
- 8) Status of patenting, if patentable, trademark or any other IPR title, if applicable:
Kaur, C., Walia S. and Pal RK. 2012. Anthocyanin rich functional products from black carrots and its process thereof. Indian patent application No. 3130/DEL/2012
- 9) Status of publication and publicity:
Khandare V, Walia S., Singh M, and Kaur C. 2011. Black carrot (*Daucus carota*) sub-sp. *sativus* juice: Processing effects on antioxidant composition and color *Food and Bio-products Processing* **89**: 482-86 (NAAS rating 7.6)

Value chain for the production of food grade nutraceuticals for use as natural antioxidants and food colorants

CPI: Dr. Suresh Walia

- 1) Title of the technology: ***Know-how for the production of capsaicinoid and capsanthin concentrates from chilli***
- 2) Commercialization status with date of licensing/ MOU:
Memorandum of Understanding (MOU) has been signed with M/s Ozone Biotech, Faridabad on March 3, 2011 for transfer of technology
- 3) Brief description of intervention/ innovation:
Chilli oleoresin is the single largest oleoresin used as natural food coloring and/or flavouring agent in the world. It is a rich source of high value capsanthin and capsaicinoids. While capsanthin concentrate is used as natural colorant in beverages, pickles, snack foods, salad dressing, seasoning and confectionery industry etc., capsaicinoid concentrate find application as flavouring agent that cause burning (hot) sensation. It also finds application in pharma industry as anti-inflammatory agent. Quality of chilli oleoresins is based on its active ingredients capsaicin, capsanthin and/or capsorubin. An improved process has been developed for production of standardized chilli oleoresins and concentrates containing food colorant –capsanthin, and pungent principles capsaicinoids. The process is simple, cost effective and involves use of food grade solvents for extraction.
- 4) Name and address of the firm(s) which has commercialized it:
M/s Ozone Biotech, Faridabad on March 3, 2011
- 5) Area (state(s)/ district(s)) covered:
M/s Ozone Biotech, Faridabad will cover Northern India
M/s Pratistha Industries, Hyderabad will cover Southern India
- 6) Volume/ quantity of Annual production and approximate sale value: NA
- 7) Benchmark (existing similar product) and Consumer acceptance, particularly in case of food products:
No such similar products are present in the Indian market.
- 8) Status of patenting, if patentable, trademark or any other IPR title, if applicable:
NA
- 9) Status of publication and publicity:
Saha S, Walia S, Kundu A, Kaur C and Sisodia R. 2014. Capsaicinoids, tocopherol and sterols content in chili (*Capsicum* sp.) by Gas chromatographic-mass spectrometric determination. *International Journal of Food Properties*. (In Press) **(NAAS rating 7.1)**



Value chain for the production of food grade nutraceuticals for use as natural antioxidants and food colorants

CPI: Dr. Suresh Walia

- 1) Title of the technology: *Extraction and production of lycopene from tomato*
- 2) Commercialization status with date of licensing/ MOU:
Memorandum of Understanding (MOU) has been signed with M/s Ozone Biotech, Faridabad on March 3, 2011 and M/s Pratistha Industries, Hyderabad on July 18, 2013 for transfer of technology
- 3) Brief description of intervention/ innovation:
Tomato is a rich source of lycopene which imparts tomato its red colour. Lycopene is an excellent natural antioxidant and a food colorant. It provides health benefits due to its significant antioxidant activity. It exhibits anticancer properties and thus used as ingredient in the preparation of Ayurvedic preparations for the control /prevention of prostate gland and other cancers, ulcers, macular degeneration etc. Lycopene find wide application as natural antioxidant and food colorant in various other herbal preparations. Bench scale know-how for the extraction of lycopene concentrate from tomato can be employed for its commercial production. The process is simple, cost effective and uses food grade solvents.
- 4) Name and address of the firm(s) which has commercialized it:
M/s Ozone Biotech, Faridabad on March 3, 2011
M/s Pratistha Industries, Hyderabad on July 18, 2013
- 5) Area (state(s)/ district(s)) covered:
M/s Ozone Biotech, Faridabad will cover Northern India
M/s Pratistha Industries, Hyderabad will cover Southern India
- 6) Volume/ quantity of Annual production and approximate sale value: NA
- 7) Benchmark (existing similar product) and Consumer acceptance, particularly in case of food products:
No such similar products are present in the Indian market.
- 8) Status of patenting, if patentable, trademark or any other IPR title, if applicable:
NA
- 9) Status of publication and publicity:
Kaur C, Walia S, Nagal S, Walia S, Singh J, Singh B, Saha S, Singh B., Kalia, P., Jaggi S and Sarika. 2013. Functional quality and antioxidant composition of selected tomato (*Solanum lycopersicon* L) cultivars grown in northern India. *LWT - Food Science and Technology*. **50**: 139–145 (NAAS rating 7.7)

Value chain for the production of food grade nutraceuticals for use as natural antioxidants and food colorants

CPI: Dr. Suresh Walia

- 1) Title of the technology: ***Know-how for the production of the blue pigment–phycocyanin from spirulina powder***
- 2) Commercialization status with date of licensing/ MOU: Memorandum of Understanding (MOU) has been signed with M/s Ozone Biotech, Faridabad on March 3, 2011
- 3) Brief description of intervention/ innovation:
The cyanobacterium *Spirulina*, a blue-green microalga is a rich source of protein (60-70%) and other nutrients. It is currently used as a health supplement and as a treatment for malnutrition. Besides being a rich source of proteins and fiber; *Spirulina* contains 10-20% of the blue pigment phycocyanin and this makes it the best natural blue color for foods. The pigment phycocyanin exhibits significant antioxidant, free radical scavenging, anti-cancer, immunomodulating, anti-inflammatory and hepatoprotective properties. Several multi-national companies are marketing *Spirulina* based algal products as food supplements. *Spirulina* powder (commercial grade) can be extracted with suitable solvent(s) and/or their blends containing one or more of other ingredients. The material after extraction can be processed in one or more steps to obtain phycocyanin concentrate. The biomass (after extraction of the pigment) can be used as protein supplements. The process is simple and cost effective.
- 4) Name and address of the firm(s) which has commercialized it:
M/s Ozone Biotech, Faridabad on March 3, 2011
- 5) Area (state(s)/ district(s)) covered:
M/s Ozone Biotech, Faridabad will cover Northern India
- 6) Volume/ quantity of Annual production and approximate sale value: NA
- 7) Benchmark (existing similar product) and Consumer acceptance, particularly in case of food products:
Comparable to the products already present in the market.
- 8) Status of patenting, if patentable, trademark or any other IPR title, if applicable:
Under preparation
- 9) Status of publication and publicity:
Kumar D, Kumar N, Pabbi S, Walia S, Dhar D W. 2013. Protocol optimization for enhanced production of natural colors in *Spirulina*. *Indian Journal of Plant Physiology* **18**: 308-312 (**NAAS rating 5.5**)

Value chain for the production of food grade nutraceuticals for use as natural antioxidants and food colorants

CPI: Dr. Suresh Walia

1) Title of the technology: ***Efficient extraction of steviol glycoside from Stevia rebaudiana leaves***

2) Commercialization status with date of licensing/ MOU:

Memorandum of Understanding (MOU) has been signed with M/s Ozone Biotech, Faridabad on March 3, 2011 and M/s Pratistha Industries, Hyderabad on July 18, 2013 for transfer of technology

3) Brief description of intervention/ innovation:

Stevia rebaudiana is a rich source of naturally occurring sweeteners-steviosides and rebaudiosides.



Unlike sugars (sucrose, glucose etc), steviol glycosides are sugar-free, low calorie natural sweeteners. Such natural sweeteners are being utilized globally in the preparation of functional foods suitable for diabetic patients. The steviol glycosides can be extracted from dried Stevia leaf powder through an efficient, simple and cost effective process through use of food grade solvents. It comprises of mainly two natural sweeteners steviosides and rebaudiosides. The process is simple, cost effective and involves use of

food grade solvents.. The natural sweetener so prepared has reduced after-bitter taste and is suitable for use in the preparation of functional foods (beverages, jams, sweets, and other processed foods replacing cane sugar. Such products are particularly useful for diabetic patients. The technology is simple, cost effective and can be demonstrated at bench scale.

4) Name and address of the firm(s) which has commercialized it:

M/s Ozone Biotech, Faridabad on March 3, 2011

M/s Pratistha Industries, Hyderabad on July 18, 2013

5) Area (state(s)/ district(s)) covered:

M/s Ozone Biotech, Faridabad will cover Northern India

M/s Pratistha Industries, Hyderabad will cover Southern India

6) Volume/ quantity of Annual production and approximate sale value: NA

7) Benchmark (existing similar product) and Consumer acceptance, particularly in case of food products:

Comparable to the products already present in the market.

8) Status of patenting, if patentable, trademark or any other IPR title, if applicable:

Under preparation

9) Status of publication and publicity:

Raina R, Bhandari S K, Romesh Chand and Sharma Y P. 2013. Causes of poor seed germination in *Stevia rebaudiana*, a low calorie sweetener. *Journal of Medicinal Plant Research* 7: 1793-1799 (NAAS rating 6.0)

Value chain for the production of food grade nutraceuticals for use as natural antioxidants and food colorants

CPI: Dr. Suresh Walia

1) Title of the technology: ***Nutraceutical enriched value added food products (beverages, candy, breads and bakery products)***

2) Commercialization status with date of licensing/ MOU:

Memorandum of Understanding (MOU) has been signed with M/s Ozone Biotech, Faridabad on March 3, 2011

3) Brief description of intervention/ innovation:

Mango pana, grapes and guava nectar: The ready-to-serve beverages available in the market are usually colored with synthetic colorants which are generally harmful to human body. Mango pana, a popular drink lack nutritional character. In order to increase its nutraceutical profile, manga pana was fortified with anthocyanin, carotenoid or phycocyanin concentrate. The novel guava, and green grape based nectars have been similarly enriched with such natural antioxidants and food colorants. Such nutraceutical RTS beverages have significant antioxidant activity and impart pleasing colour, stability and health promoting capacity.



4) Name and address of the firm(s) which has commercialized it:

NA

5) Area (state(s)/ district(s)) covered:

M/s Ozone Biotech, Faridabad will cover Northern India

6) Volume/ quantity of Annual production and approximate sale value: NA

7) Benchmark (existing similar product) and Consumer acceptance, particularly in case of food products:

No such similar products are present in the Indian market.

8) Status of patenting, if patentable, trademark or any other IPR title, if applicable:

NA

9) Status of publication and publicity:

NA

Value chain for the production of food grade nutraceuticals for use as natural antioxidants and food colorants

CPI: Dr. Suresh Walia

- 1) Title of the technology : ***Nutraceutical enriched value added food products (beverages, candy, breads and bakery products)***
- 2) Commercialization status with date of licensing/ MOU:
Not yet done
- 3) Brief description of intervention/ innovation:
Aonla candy: Amla fruit is known for its pharmaceutical applications in Ayurvedic system. It is a rich source of ascorbic acid and other phenolics. During conversion of raw aonla to candy, significant amount of its natural ingredients is lost during processing. Incorporation of natural red anthocyanin or blue phycocyanins provides stability and high sensory appeal to the amla products. Anthocyanin and phycocyanin enriched aonla candy thus combines the biologically active components from aonla, black carrot and Spirulina.
- 4) Name and address of the firm(s) which has commercialized it:
NA
- 5) Area (state(s)/ district(s)) covered:
NA
- 6) Volume/ quantity of Annual production and approximate sale value: NA
- 7) Benchmark (existing similar product) and Consumer acceptance, particularly in case of food products:
No such similar products are present in the Indian market.
- 8) Status of patenting, if patentable, trademark or any other IPR title, if applicable:
NA
- 9) Status of publication and publicity:
NA



Value chain for the production of food grade nutraceuticals for use as natural antioxidants and food colorants

CPI: Dr. Suresh Walia

- 1) Title of the technology : ***Nutraceutical enriched value added food products (beverages, candy, breads and bakery products)***
- 2) Commercialization status with date of licensing/ MOU:
Not yet done
- 3) Brief description of intervention/ innovation:
Breads biscuits and bakery products: Carrot concentrates, jamun concentrate, sweet and pungent capsicum concentrate and dehydrated capsicum carrot powder/pomace have been successfully used as functional ingredients to improve nutritional status of wheat breads and other bakery products. Health-promoting ingredients (nutraceuticals) such as anthocyanins, carotenoids, phycocyanins can also be incorporated in such functional foods to impart characteristic purple, golden and pink hues, and higher antioxidant activity and sensory scores.



- 4) Name and address of the firm(s) which has commercialized it:
NA
- 5) Area (state(s)/ district(s)) covered:
NA
- 6) Volume/ quantity of Annual production and approximate sale value: NA
- 7) Benchmark (existing similar product) and Consumer acceptance, particularly in case of food products:
No such similar products are present in the Indian market.
- 8) Status of patenting, if patentable, trademark or any other IPR title, if applicable:
Kaur, C., Walia, S., Nath, P., Saha, S. 2012. Carotenoid rich composition and process of its preparation. Indian patent application No. 909/DEL/2012
- 9) Status of publication and publicity:
NA

Value chain for the production of food grade nutraceuticals for use as natural antioxidants and food colorants

CPI: Dr. Suresh Walia

- 1) Title of the technology : ***Nutraceutical enriched value added food products (beverages, candy, breads and bakery products)***
- 2) Commercialization status with date of licensing/ MOU:
Not yet done
- 3) Brief description of intervention/ innovation:

Nutraceutical enriched salsa comprising of colored capsicum and tomato: During the peak season there is a huge glut of tomato and capsicum because of over production and farmers do not fetch the premium price. Red capsicum and tomato can be value processed into a delicious salsa sauce which has a rich red color and a unique flavour. Capsicum-tomato salsa is a rich blend of health promoting antioxidants such as carotenoids, phenolics and vitamin C and has higher



nutrition value than even the popular tomato sauce. It is high in capsanthin and lycopene and can be valuable product for combating vitamin A deficiency in India. Salsa is ideal for consumption as bread spread, pizza sauce and topping base. Freshly harvested red capsicum and tomato can be processed for

developing a salsa, a thick sauce like product. Spices, flavors and other minor ingredients can be added depending upon the desired taste.

- 4) Name and address of the firm(s) which has commercialized it:
NA
- 5) Area (state(s)/ district(s)) covered:
NA
- 6) Volume/ quantity of Annual production and approximate sale value: NA
- 7) Benchmark (existing similar product) and Consumer acceptance, particularly in case of food products:
No such similar products are present in the Indian market.
- 8) Status of patenting, if patentable, trademark or any other IPR title, if applicable:
Kaur, C., Walia, S., Nath, P., Saha, S. 2012. Carotenoid rich composition and process of its preparation. Indian patent application No. 909/DEL/2012
- 9) Status of publication and publicity:
NA